Harvard University

Library of

The Medical School

and

The School of Public Health

VE RI TAS

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY

THE UNIVERSITY OF LIVERPOOL

ANNALS
OF
TROPICAL MEDICINE AND PARASITOLOGY

ISSUED BY THE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Edited by
PROFESSOR J. W. W. STEPHENS, M.D.Cantab., D.P.H.
PROFESSOR R. NEWSTEAD, M.Sc., J.P., F.R.S., A.L.S., F.E.S., Hon. F.R.H.S.
PROFESSOR WARRINGTON YORKE, M.D.
AND
PROFESSOR SIR RONALD ROSS, K.C.B., F.R.S., M.D., F.R.C.S.
MAJOR I.M.S. (RET.)

Editorial Secretary
DR. H. B. FANTHAM

VOLUME VIII
(April 21, 1914, to January 29, 1915)

With Frontispiece, thirty-one plates, seventeen figures in text, three maps, and thirty-three charts

LIVERPOOL:
AT THE UNIVERSITY PRESS, 57 ASHTON STREET

Editorial Secretary
Dr. H. B. FANTHAM,
School of Tropical Medicine,
The University,
Liverpool.

CONTENTS

No. 1. April 21, 1914

PAGE

Yorke, Warrington; and Blacklock, B.

The Differentiation of the more Important Mammalian Trypanosomes. Plate I 1

Taylor, J. A.

Bronchial Spirochaetosis in Uganda, with Pneumonic Symptoms 13

Thomson, David.

Attempts to find Disease Germs in the European Bed Bug (*Cimex lectularius*) after Feeding Experiments in Various Diseases: Leprosy, Lymphadenoma, Carcinoma, etc. 19

Macfie, J. W. Scott.

On the Pathogenicity of the Trypanosome (*T. nigeriense*) from a Case of Sleeping Sickness from Eket, Southeren Nigeria. 29

Macfie, J. W. Scott; and Johnston, J. E. L. ...

A Note on the Occurrence of Spirochaetosis of Fowls in Southern Nigeria. Plates II, III 41

Yorke, Warrington; and Blacklock, B.

Observations on a so-called Cure for Trypanosomiasis... 51

Yorke, Warrington; and Blacklock, B.

Antimony Trioxide in the Treatment of Experimental Trypanosomiasis ... 55

Johnston, J. E. L.

Observations on Variations in Form of Microfilariae Found in Man. Plate IV 73

Seidelin, Harald; and Connal, Andrew.

A Note upon the Occurrence of a *Plasmodium* in the Blood of West African Monkeys 81

Lloyd, Llewellyn.

Note on Scratching Birds and Tsetse-Fly 83

Thomson, David.

The Origin and Development of Gametes (Crescents) in Malignant Tertian Malaria: Some Observations on Flagellation, etc. Plate V 85

Johnston, T. Harvey.

Second Report on the Cestoda and Acanthocephala Collected in Queensland. Plate VI 105

Priestley, Henry.

Microsporon scorteum, n.sp., from a Case of Ringworm in Man. Plate VII 113

Stephens, J. W. W.

A New Malaria Parasite of Man. Plates VIII—X 119

Christopherson, J. B.

The Mutilation of Wounded on the Battlefield: A Case Described. Plate XI 129

CONTENTS

No. 2. July 22, 1914

PAGE

JAMES, WILLIAM M.

A Study of the *Entamoebae* of Man in the Panama Canal Zone. Plates XII—XVII 133

DEEKS, W. E.

Dysenteries in the Canal Zone, with Special Reference to Amoebic Dysentery 321

DEEKS, W. E.

Emetine in Amoebic Dysentery 353

DEEKS, W. E.

Six Day Fever in Panama 357

MARZINOWSKY, E. L.

Communication from the Commission for the Study of Malaria in Russia ... 367

CONTENTS

No. 3. December 15, 1914

PAGE

O'BRIEN, J. M.

A Study of Some Cases of Yellow Fever in Guayaquil, Ecuador. Plate XVIII 369

MACFIE, J. W. SCOTT; and GALLAGHER, G. H.

Sleeping Sickness in the Eket District of Nigeria. Plates XIX—XXIII, and Map 379

MACFIE, J. W. SCOTT.

Notes on Some Blood Parasites Collected in Nigeria. Plates XXIV, XXV ... 439

TODD, JOHN L.

The Trypanosome of Gambian Mice 469

FANTHAM, H. B.

The Granule Phase of Spirochaetes 471

CHRISTOPHERSON, J. B.

On a Case of *Naso-Oral Leishmaniasis* (Corresponding to the Description of *Espundia*); and on a Case of *Oriental Sore*, both Originating in the Anglo-Egyptian Sudan. Plates XXVI, XXVII 485

SCHWETZ, LE DR. J.

Quelques observations préliminaires sur la Morphologie et la Biologie de la Larve, de la Nymphe et de l'Image de l'*Auchmeromyia luteola*, Fabr. ... 497

CONTENTS. No. 3—*continued*

PAGE

BARRATT, J. O. WAKELIN; and YORKE, WARRINGTON.
The Relation of Bile Pigments to Haemoglobin 509

HATORI, DR. J.
The Anti-Malarial Campaign in Formosa. With Map 537

SEIDELIN, HARALD.
Klossiella sp. in the Kidney of a Guinea-pig. Plates XXVIII, XXIX ... 553

BREINL, A.; and PRIESTLEY, H.
Changes in the Neutrophile Blood-Picture of Arneth observed in Children Living in Tropical Queensland 565

BREINL, A.; YOUNG, W. J.
The Occurrence of Lead Poisoning amongst North Queensland Children 575

BREINL, A.; PRIESTLEY, H.
Observations on the Blood Conditions of Children of European Descent residing in Tropical Australia 591

NICOLL, WILLIAM.
On the Migration of the Larvae of *Onchocerca gibsoni* through the Capsule of the Worm Nodule 609

FANTHAM, H. B.; and PORTER, ANNIE.
The Morphology, Biology and Economic Importance of *Nosema bombi*, n.sp., Parasitic in various Humble Bees (*Bombus* spp.). Plate XXX 623

STEPHENS, J. W. W.
Studies in Blackwater Fever. II. A Schedule for Recording Cases of Blackwater Fever 639

CONTENTS

No. 4. January 29, 1915

PAGE

HATORI, J.
Blackwater Fever in Formosa. With Map 641

WHYTE, G. DUNCAN.
Splenomegaly: Being a Report on a Hundred and Four Cases met with in South China 659

BAHR, PHILIP H.
Notes on Yaws in Ceylon, with Special Reference to its Distribution in that Island and its Tertiary Manifestation. Plate XXXI 675

Yours faithfully
T. Spencer Cobbold

Volume VIII April, 1914 No. 1

ANNALS
OF
TROPICAL MEDICINE AND PARASITOLOGY

ISSUED BY

THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Edited by
PROFESSOR J. W. W. STEPHENS, M.D. Cantab., D.P.H.
PROFESSOR R. NEWSTEAD, M.Sc., J.P., F.R.S., A.L.S., F.E.S., Hon. F.R.H.S.
PROFESSOR WARRINGTON YORKE, M.D.
AND
PROFESSOR SIR RONALD ROSS, K.C.B., F.R.S., M.D., F.R.C.S.,
MAJOR I.M.S. (RET.)

Editorial Secretary
DR. H. B. FANTHAM,
School of Tropical Medicine,
The University,
Liverpool.

C. Tinling & Co., Ltd.
Printers to the University Press of Liverpool
53 Victoria Street

THE INCORPORATED
LIVERPOOL SCHOOL OF TROPICAL MEDICINE

FOUNDED BY SIR ALFRED LEWIS JONES, K.C.M.G.
(Affiliated with the University of Liverpool)

Hon. President: H.R.H. PRINCESS CHRISTIAN OF SCHLESWIG-HOLSTEIN.
Chairman: MR. F. C. DANSON.
Vice-Chairman: Professor R. CATON.
Hon. Vice-Presidents: THE DUKE OF NORTHUMBERLAND, K.G.
THE EARL OF DERBY, G.C.V.O.
EARL CROMER, G.C.B.
VISCOUNT MILNER, G.C.B.
LORD PIRRIE, K.P.
SIR OWEN PHILIPPS, K.C.M.G.
SIR EDWIN DURNING LAWRENCE, Bart.
SIR WILLIAM H. LEVER, BART.
MR. O. HARRISON WILLIAMS

COMMITTEE

Mr. H. J. READ, C.M.G.	*Colonial Office*
Vice-Chancellor SIR ALFRED DALE	*University of Liverpool*
Sir W. B. BOWRING, Bart.	*Council of University of Liverpool*
Professor R. CATON	
Professor W. HERDMAN, F.R.S.	*Senate of University of Liverpool*
Professor J. M. BEATTIE	
Dr. C. J. MACALISTER	*Royal Southern Hospital*
Mr. G. P. NEWBOLT	
Mr. C. BOOTH (Jun.)	*Steamship Owners' Association*
Mr. T. F. HARRISON	
Mr. A. R. MARSHALL	*Shipowners' Association*
Mr. W. ROBERTS	
Mr. J. W. ALSOP	
Mr. G. BROCKLEHURST	
Mr. C. LIVINGSTON	
Mr. J. W. W. DANSON	
Professor E. W. HOPE	
Mr. R. RANKIN	
Professor E. GLYNN	
Mr. E. G. BUCKLEY	
Mr. A. L. REA, *Hon. Treasurer*	
Mr. A. H. MILNE, C.M.G., *Secretary.*	

B 10 Exchange Buildings, Liverpool

Staff, 1914

1. At the University of Liverpool

Professors -	JOHN WILLIAM WATSON STEPHENS, M.D., Cantab., D.P.H. *Sir Alfred Jones Professor of Tropical Medicine* ROBERT NEWSTEAD, F.R.S., M.Sc., A.L.S., F.E.S., *Dutton Memorial Professor of Entomology* MAJOR SIR RONALD ROSS, K.C.B., F.R.S., LL.D., F.R.C.S., D.P.H., M.D., D.Sc. Nobel Laureate 1902. (Indian Medical Service, retired). *Professor of Tropical Sanitation* WARRINGTON YORKE, M.D., *Walter Myers Professor in Parasitology*
Lecturers -	HAROLD BENJAMIN FANTHAM, D.Sc., B.A., *Lecturer in Parasitology* HENRY FRANCIS CARTER, S.E.A.C. DIPL., F.E.S., *Lecturer in Entomology* WILLIAM THOMAS PROUT, M.B., C.M.G., *Lecturer on Tropical Sanitation* HAROLD WOLFERSTAN THOMAS, M.D., C.M. PROF. E. W. HOPE, M.D., D.Sc., *Lecturer on Municipal Sanitation*
Honorary Lecturer -	MAJOR JOSEPH FITZGERALD BLOOD, M.D., M.Ch. (Indian Medical Service, retired)
Honorary Statistician	WALTER STOTT
Bibliographer -	WALTER DRAWZ

2. At the Yellow Fever Bureau

HARALD SEIDELIN, M.D. (Scientific Secretary).

3. At the Runcorn Research Laboratories

Director -	B. BLACKLOCK, M.D., D.P.H.
Assistant -	Vacant.

4. At the Royal Southern Hospital, Liverpool

Physicians -	CHARLES JOHN MACALISTER, M.D., F.R.C.P. JOHN LLOYD ROBERTS, M.D., M.R.C.P.
Surgeons -	D. DOUGLAS CRAWFORD, F.R.C.S. ROBERT JONES, F.R.C.S., Ch.M. GEORGE PALMERSTON NEWBOLT, M.B., F.R.C.S.
Tropical Pathologist -	JOHN WILLIAM WATSON STEPHENS, M.D. Cantab.

5. On Expedition

HAROLD WOLFERSTAN THOMAS, M.D. C.M.

Laboratory
JOHNSTON LABORATORY, UNIVERSITY OF LIVERPOOL

Research Laboratory:
RUNCORN

Hospital:
ROYAL SOUTHERN HOSPITAL, LIVERPOOL

Secretary's Office:
B 10, EXCHANGE BUILDINGS, LIVERPOOL

NOTICE

The following courses of instruction will be given by the Liverpool School of Tropical Medicine during 1914:—

Full Course begins 6 January. Advanced Course begins 1 June.
Diploma Examination, 6 April. Certificate Examination, 30 June.
Full Course begins 15 September.
Diploma Examination, 14 December.
These dates are subject to revision.

The full Course of Instruction is open to all qualified medical men, and the Examination to all students who have taken out this full course.

Fee for the full Course of Instruction—Thirteen Guineas.

Fee for the Diploma Examination—Five Guineas.

Fee for the Short Course of Instruction—Four Guineas.

Fee for the use of a School microscope during one term—Ten shillings and sixpence.

For prospectus and further information, application should be made to the Dean of the Medical Faculty, University of Liverpool.

The following have obtained the Diploma in Tropical Medicine of the University of Liverpool:—

Diploma in Tropical Medicine

Date of Diploma	
1904	Augustine, Henry Joshua
1904	Bennett, Arthur King
1904	Bruce, William James
1904	Byrne, John Scott
1904	Clayton, Thomas Morrison
1904	Dalziel, John McEwen
1904	Dee, Peter
1904	Greenidge, Oliver Campbell
1904	Hehir, Patrick
1904	Khan, Saiduzzafor
1904	Laurie, Robert
1904	Maclurkin, Alfred Robert
1904	McConnell, Robert Ernest
1904	Nicholson, James Edward
1904	Philipson, Nicholas
1904	Sharman, Eric Harding
1904	Thomson, Frank Wyville
1904	Walker, George Francis Clegg
1905	Anderson, Catherine Elmslie
1905	Brown, Alexander
1905	Caldwell, Thomas Cathcart
1905	Critien, Attilio
1905	Hooton, Alfred
1905	Hudson, Charles Tilson
1905	Illington, Edmund Moritz
1905	Macfarlane, Robert Maxwell
1905	Maddock, Edward Cecil Gordon
1905	Moore, James Jackson
1905	Nightingale, Samuel Shore
1905	Radcliffe, Percy Alexander Hurst
1905	Young, John Cameron
1906	Adie, Joseph Rosamond
1906	Arnold, Frank Arthur
1906	Bate, John Brabant
1906	Bennetts, Harold Graves
1906	Carter, Robert Markham
1906	Chisholm, James Alexander
1906	Clements, Robert William
1906	Dundas, James
1906	Faichnie, Norman
1906	Jeffreys, Herbert Castelman
1906	Mackenzie, Donald Francis
1906	Pailthorpe, Mary Elizabeth
1906	Palmer, Harold Thornbury
1906	Pearse, Albert
1906	Sampey, Alexander William
1906	Smithson, Arthur Ernest
1906	Taylor, Joseph van Someron
1906	Taylor, William Irwin
1906	Tynan, Edward Joseph

Date of Diploma	
1906	Watson, Cecil Francis
1906	Willcocks, Roger Durant
1906	Williamson, George Alexander
1907	Allan, Alexander Smith
1907	Allwood, James Aldred
1907	Bond, Ashton
1907	Branch, Stanley
1907	Collinson, Walter Julius
1907	Davey, John Bernard
1907	Donaldson, Anson Scott
1907	Fell, Matthew Henry Gregson
1907	Gann, Thomas William Francis
1907	Graham, James Drummond
1907	Hiscock, Robert Carroll
1907	Keane, Joseph Gerald
1907	Kennan, Richard Henry
1907	Kenrick, William Hamilton
1907	Le Fanu, George Ernest Hugh
1907	Mackey, Charles
1907	Maddox, Ralph Henry
1907	McCarthy, John McDonald
1907	Raikes, Cuthbert Taunton
1907	Ryan, Joseph Charles
1907	Vallance, Hugh
1908	Caverhill, Austin Mack
1908	Crawford, Gilbert Stewart
1908	Dalal, Kaikhusroo Rustomji
1908	Dansey-Browning, George
1908	Davidson, James
1908	Dickson, John Rhodes
1908	Dowdall, Arthur Melville
1908	Glover, Henry Joseph
1908	Greaves, Francis Wood
1908	Goodbody, Cecil Maurice
1908	Harrison, James Herbert Hugh
1908	Joshi, Lemuel Lucas
1908	Le Fanu, Cecil Vivian
1908	Luethgen, Carl Wilhelm Ludwig
1908	Mama, Jamshed Byramji
1908	McCay, Frederick William
1908	McLellan, Samuel Wilson
1908	Pearce, Charles Ross
1908	Schoorel, Alexander Frederik
1908	Smith, John Macgregor
1908	Stewart, George Edward
1908	Tate, Gerald William
1908	Whyte, Robert
1909	Abercrombie, Rudolph George
1909	Allin, John Richard Percy
1909	Armstrong, Edward Randolph
1909	Barrow, Harold Percy Waller
1909	Beatty, Guy
1909	Carr-White, Percy
1909	Chevallier, Claude Lionel
1909	Clark, William Scott
1909	Cope, Ricardo
1909	Fleming, William
1909	Hanschell, Hother McCormick
1909	Hayward, William Davey
1909	Henry, Sydney Alexander
1909	Innes, Francis Alexander

Date of Diploma	
1909	Jackson, Arthur Frame
1909	Kaka, Sorabji Manekji
1909	McCabe-Dallas, Alfred Alexander Donald
1909	Meldrum, William Percy
1909	Murphy, John Cullinan
1909	Samuel, Mysore Gnananandaraju
1909	Shroff, Kawasjee Byramjee
1909	Thornely, Michael Harris
1909	Turkhud, Violet Ackroyd
1909	Webb, William Spinks
1909	Yen, Fu-Chun
1910	Brabazon, Edward
1910	Castellino, Louis
1910	Caulcrick, James Akilade
1910	Dowden, Richard
1910	Haigh, William Edwin
1910	Hamilton, Henry Fleming
1910	Hefferman, William St. Michael
1910	Hipwell, Abraham
1910	Homer, Jonathan
1910	Houston, William Mitchell
1910	James, William Robert Wallace
1910	Johnstone, David Patrick
1910	Korke, Vishnu Tatyaji
1910	Macdonald, Angus Graham
1910	Macfie, John Wm. Scott
1910	Manuk, Mack Walter
1910	Murison, Cecil Charles
1910	Nanavati, Kishavlal Balabhai
1910	Nauss, Ralph Welty
1910	Oakley, Philip Douglas
1910	Pratt, Ishmael Charles
1910	Sabastian, Thiruchelvam
1910	Shaw, Hugh Thomas
1910	Sieger, Edward Louis
1910	Sousa, Pascal John de
1910	Souza, Antonio Bernardo de
1910	Waterhouse, John Howard
1910	White, Maurice Forbes
1911	Blacklock, Breadalbane
1911	Brown, Frederick Forrest
1911	Chand, Diwan Jai
1911	Holmes, John Morgan
1911	Ievers, Charles Langley
1911	Iles, Charles Cochrane
1911	Ingram, Alexander
1911	Kirkwood, Thomas
1911	Knowles, Benjamin
1911	Liddle, George Marcus Berkeley
1911	Lomas, Emanuel Kenworthy
1911	Mackarell, William Wright
1911	MacKnight, Dundas Simpson
1911	Mascarenhas, Joseph Victor
1911	Murray, Ronald Roderick
1911	Oluwole, Akidiya Ladapo
1911	Rao, Koka Ahobala
1911	Sinton, John Alexander
1911	Tarapurvalla, Byramji Shavakshah
1911	Taylor, John Archibald
1911	Woods, William Medlicott

Date of Diploma	
1912	Aeria, Joseph Reginald
1912	Anderson, Edmund Litchfield
1912	Borle, James
1912	Bowie, John Tait
1912	Brassey, Laurence Percival
1912	Christie, David
1912	Dillon, Henry de Courcy
1912	Dunn, Lillie Eleanor
1912	Hardwicke, Charles
1912	Jagose, Jamshed Rustomji
1912	Kochhar, Mela Ram
1912	McGusty, Victor William Tighe
1912	Milne, Arthur James
1912	Mitra, Manmatha Nath
1912	Myles, Charles Duncan
1912	Pelly, Huntly Nevins
1912	Prasad, Bindeshwari
1912	Prentice, George
1912	Ross, Frank
1912	Russell, Alexander James Hutchison
1912	Ruthven, Morton Wood
1912	Sandilands, John
1912	Seddon, Harold
1912	Smalley, James
1912	Strickland, Percy Charles Hutchison
1912	Watson, William Russel
1913	Austin, Charles Miller
1913	Banker, Shiavux Sorabji
1913	Becker, Johann Gerhardus
1913	Carrasco, Milton
1913	Clark, James McKillican
1913	Forsyth, Charles
1913	Grahame, Malcolm Claude Russell
1913	Grieve, Kelburne King

Date of Diploma	
1913	Hargreaves, Alfred Ridley
1913	Hepper, Evelyn Charles
1913	Hiranand, Pandit
1913	Jackson, Oswald Egbert
1913	Khaw, Ignatius Oo Kek
1913	MacKelvie, Maxwell
1913	MacKinnon, John MacPhail
1913	Macmillan, Robert James Alan
1913	Mouat-Biggs, Charles Edward Forbes
1913	Noronha, John Carmel
1913	O'Connor, Edward
1913	Olubomi-Beckley, Emanuel
1913	Pestonji, Ardeshir Behramshah
1913	Puttanna, Dodballapur Sivappa
1913	Reford, John Hope
1913	Smith, Edward Arthur
1913	Stewart, Samuel Dudley
1913	Walker, Frederick Dearden
1913	Wilbe, Ernest Edward
1913	Wilson, Hubert Francis
1913	Yin, Ulg Ba
1913	Young, William Alexander
1914	Arculli, Hassan el
1914	Chohan, Noormahomed Kasembhai
1914	Connell, Harry Bertram
1914	Gwynne, Joseph Robert
1914	Hodkinson, Samuel Paterson
1914	Jackson, Arthur Ivan
1914	Kaushash, Ram Chander
1914	Kelsall, Charles
1914	Luanco y Cuenca, Maximino
1914	Rowe, John Joseph Stephen
1914	Sur, Sachindra Nath
1914	Wilkinson, Arthur Geden

EDITORIAL NOTICE

By order of the Committee of the Incorporated Liverpool School of Tropical Medicine, the series of the Reports of the School, which had been issued since 1899, were followed, from January 1, 1907, by the Annals of Tropical Medicine and Parasitology, of which this is the first number of the eighth volume.

Altogether twenty-one Memoirs, besides other works, were published by the School since 1899, and of these ten, containing 519 quarto or octavo pages and 95 plates and figures, were published during the two years 1904 and 1905.

The Annals are issued by the Committee of the School, and will contain all such matter as was formerly printed in the Reports—that is to say, accounts of the various expeditions of the School and of the scientific work done in its laboratories at the University of Liverpool and at Runcorn. In addition, however, to School work, original articles from outside on any subject connected with Tropical Medicine and Hygiene, and Parasitology may be published if found suitable (see notice on back of cover); so that, in all probability, not less than four numbers of the Annals will be issued annually. Each number will be brought out when material sufficient for it has been accumulated.

THE DIFFERENTIATION OF THE MORE IMPORTANT MAMMALIAN TRYPANOSOMES

BY

WARRINGTON YORKE

AND

B. BLACKLOCK

(From the Runcorn Research Laboratories of the Liverpool School of Tropical Medicine)

(Received for publication October 6, 1913)

PLATE I

INTRODUCTION

Notwithstanding the very considerable number of recent papers dealing with this subject, there is still much confusion concerning the identity of many of the trypanosomes which have been described. The difficulties which confront anyone who, not having had considerable practical experience, endeavours to assign to its proper position any trypanosome which he may encounter, are in many cases almost insuperable. Even where he can readily obtain access to the enormous literature on the subject he is left in perplexity, owing to the diversity of opinions expressed by various authors as to the relative value of particular methods of differentiation. It appears to us that it may be of service to review the methods at present employed in the effort to differentiate species, and to indicate those which we have found to be of practical value. During a number of years we have had opportunity of examining trypanosomes both in Africa and at Runcorn, where at the present time fifteen strains are maintained.

A given trypanosome may present characteristic features either in the vertebrate or in the invertebrate host, if such exist, and must therefore be examined in both of these where possible. First we shall review the methods of identification which can be applied to the parasite as it appears in the vertebrate and then those which can be applied to it in the invertebrate host.

VERTEBRATE HOST

I. MOTILITY

Although certain trypanosomes, e.g., *T. vivax* and *T. lewisi*, as a rule move more rapidly than others across the microscope field, yet it is known that many factors such as the condition of the infected animal, the lapse of time between making and examining the film, and the temperature at which the examination is conducted, have an important influence on the motility of the parasites, so that one frequently fails to observe that activity which is considered characteristic of them.

II. MORPHOLOGY

(A) *Examination of fresh preparations*

In our experience conclusions as to morphology, derived from examination of fresh films, are untrustworthy as a means of differentiating species. In view of the relatively meagre information which can be obtained from such, in comparison with that which is derived from stained preparations, we are of opinion that for practical purposes no value should be attached to this method.

(B) *Examination of stained preparations*

We shall first consider those characters which can be recognised by simple microscopic examination, i.e., *Qualitative characters*, and afterwards those which require mathematical expression for their appreciation, i.e., *Quantitative characters.*

(*a*) *Qualitative characters*

1. The Flagellum. The character of this structure is of great importance as an aid to identification of trypanosome species, and is the basis of Laveran's classification. This author arranges trypanosomes in three groups, according to whether all the individuals have a free flagellum, none have a free flagellum, or some have a free flagellum while others have not. This character is of great value for the general grouping of trypanosomes.

2. The Blepharoplast. This is present and distinct in all known pathogenic species, except in the single instance of *T. equinum*, where it is absent or, at any rate, extremely small.

The position, size, and shape of the blepharoplast are variable. As a rule, it is rounded and may be situated terminally, laterо-terminally, or more frequently centrally near the posterior end. In rare cases, e.g., *T. transvaaliense*, *T. ingens* and *T. tragelaphi*, it lies only slightly posterior to the nucleus in the centre of the body. Although the position of the blepharoplast may be of some assistance in determining species, e.g., *T. simiae* (*T. ignotum*), where it frequently projects laterally as a small excrescence, it is doubtful whether position is, in itself, of any diagnostic value except in the case of the three trypanosomes mentioned above. On the other hand, the size and shape of the blepharoplast may be of considerable assistance. For example, the large egg-shaped blepharoplast of *T. cruzi* is characteristic, as is similarly the transversely lying rod-shaped blepharoplast of *T. lewisi*.

3. The Nucleus. The shape, size, compactness, or diffuseness of the nucleus are, as a rule, of no assistance. Mention may, however, be made of the transversely lying band-shaped nucleus of such large species as *T. ingens*. The position, on the other hand, may be of some service in differentiating one species from another. In the case of *T. lewisi* the nucleus is so invariably anterior to the middle of the body as to be characteristic. Again, the fact that certain individuals of some polymorphic trypanosomes exhibit a posterior position of the nucleus, has been used for the purpose of subdividing the trypanosomes of Group III. Rarely individuals are encountered in which no nucleus is visible, but as this phenomenon probably occurs in all strains it is of no diagnostic value. It must be clearly understood, however, that slight displacements of the nucleus either in the anterior or posterior direction are found in individuals of all species, and are hence of no diagnostic significance.

4. Cytoplasm. Many attempts have been made to discover any features in the cytoplasm of trypanosomes which might enable us to separate one species from another.

Staining. The staining reaction of the cytoplasm depends so much on the technique adopted that it is of little value in the differentiating of species, although it is generally recognised that all things being equal *T. lewisi* stains more faintly than the common pathogenic trypanosomes.

Posterior extremity. Emphasis has been laid upon the character of the posterior extremity as being of diagnostic value. In the case of *T. equiperdum*, Laveran and Mesnil consider that a truncated or notched posterior extremity is typical. Again, in *T. rhodesiense*, forms in which the posterior end is prolonged, 'snout forms,' are mentioned by Stephens and Fantham as peculiar to this species, while others regard a notched appearance of the posterior end of certain forms as characteristic of the same species.

Vacuoles and granules. Vacuoles and granules in the cytoplasm have also been held to be of diagnostic value, e.g., the precentrosomic vacuole described by Laveran and Mesnil in *T. equiperdum*, and the arrangement of granules in *T. brucei* described by MacFadyean as distinguishing this species from *T. evansi.*

Such features as the above have proved so inconstant in the species mentioned, and have so frequently been observed in other species, that we find them valueless as diagnostic characters.

General form. Of some value is the general shape of the creature. For instance *T. vivax* is, as a rule, more or less club-shaped, the greatest breadth being posterior to the nucleus, and the body gradually tapering towards the anterior extremity. Gross differences in length or in width are undoubtedly of great value. Thus a parasite like *T. ingens* is at a glance distinguished from most mammalian trypanosomes on account of its remarkable size. Similarly, *T. montgomeryi* can be distinguished from *T. congolense* and *T. nanum* by its markedly greater breadth, and the Royal Society Commission have differentiated *T. caprae* from *T. vivax* by means of the heavier build of the former.

Undulating membrane. We have not found the character of this to be *per se* of any particular value in distinguishing one species from another.

Myonemes. Amongst mammalian trypanosomes these appear to be limited to certain of the larger species, such as *T. ingens.*

Having discussed the diagnostic value of the various characters which can be recognised by simple microscopic examination we proceed to consider those which necessitate the employment of more precise technique for their recognition.

(*b*) *Quantitative characters*

The size of the trypanosome is of great assistance in distinguishing one species from another, and must be ascertained before one is justified in identifying a parasite. As many individuals as possible should be measured, and the maximum, minimum and average lengths of the parasite determined. We consider it of greater importance to find out the maximum and minimum lengths, or, in other words, the range of variation in size of the individuals, than to attempt to determine the average length. In monomorphic trypanosomes, where the range of variation in size is small, the various records of the average length of the parasite are found to agree, but in the case of the polymorphic trypanosomes, where the largest form may be 30μ longer than the shortest, ascertaining the average length is, for reasons which we will discuss later, of comparatively little value.

Besides the length of a trypanosome, the breadth must also be measured. Although in the majority of cases this will prove to be of little value, yet it is the only way in which certain species can be distinguished, e.g., *T. caprae* from *T. vivax*, and *T. montgomeryi* from *T. congolense*. We have not found it of any value to measure in detail the various portions of trypanosomes, such as the size of the nucleus, the distance of blepharoplast from posterior extremity and so on, as the variations within the same species are great.

Recently considerable work has been done in the attempt to differentiate species by the use of curves showing the percentage distribution of individuals according to length. It was hoped that in this way it would be possible graphically to distinguish any one species from another. To a certain extent only has this hope been realised. In the case of such monomorphic trypanosomes as *T. congolense*, *T. nanum* and *T. vivax*, the curves made by various observers correspond fairly closely, and may therefore be regarded as characteristic. No such close correspondence, however, is observable in the curves of polymorphic trypanosomes. As illustrative of this, we cannot do better than consider *T. rhodesiense*. A large number of graphs based on the measurement of at least 1,000 individuals has been constructed by various workers, using either the same or different strains of the trypanosome. Stephens and Fantham made two such curves. Kinghorn and Yorke are responsible for another, whilst the Royal Society Commission has constructed five curves from various strains of the parasite in Nyasaland. Taute has also more recently constructed a similar curve. On comparing these graphs, one is at once impressed by the extraordinary variation they exhibit. In order to explain this diversity of appearance, we have made a careful study of the various factors which might influence the character of the graphs. In examining the blood of animals infected with such trypanosomes as *T. rhodesiense*, one is struck with the fact that whilst on some days all forms of parasites are seen, on others the vast majority of the individuals present corresponds to one particular type, either the long free flagellated, or the short aflagellar form. It is this fact which seems to us to lie at the root of the matter. Under such circumstances it is obvious that if the majority of the preparations selected for measurement happened to be made on those days when the short form greatly preponderated then the apex of the graph would be displaced towards the left (short end), whilst on the other hand if the long form should happen to predominate, then the character of the curve would be radically altered and the peak displaced to the right (long end). In our opinion, it does not matter whether films are taken from one animal during ten days of the disease, or from ten animals on one day of the disease and 100 in each measured, the resulting curves must show great variation. So much so that if they are at all similar, it would appear to be chiefly a matter of coincidence.

The work of Miss Robertson on the endogenous cycle of *T. gambiense* and another polymorphic parasite from Uganda offers an explanation of the variations in the type of trypanosome occurring in an animal on different days of the infection. Until the factors governing this periodic variation in type are thoroughly understood, it is obviously futile to attempt to diagnose one polymorphic trypanosome from another by measuring samples taken haphazard or on a number of consecutive days.

Other factors such as the particular technique adopted, and the species of animal or animals employed, although doubtless exerting some influence on the character of the graphs obtained, are of but little importance in comparison with that just discussed.

In conclusion, therefore, we are of opinion that in so far as polymorphic trypanosomes are concerned the character of biometric curves affords no assistance in diagnosis. In fact, the only valuable information to be obtained from measuring polymorphic trypanosomes is the range of variation in size of the individuals, or in other words, the maximum and minimum lengths.

III. PATHOGENICITY

The manner in which trypanosomes affect different laboratory animals has in the past been considered to be of great use in the diagnosis of species; but the accumulated evidence of recent years has shown that this is by no means so reliable a criterion as was formerly thought. This especially refers to what may be called quantitative reactions, i.e., duration of the disease in a series of animals infected with any given trypanosome. It is now a well-recognised fact that passage of a trypanosome through a series of animals of one species may greatly increase the virulence of the parasite for that particular kind of animal. As all the strains kept at the present time in European laboratories are maintained by direct inoculation from animal to animal, their virulence has frequently been artificially increased. An example of this is seen in the case of *T. gambiense.* The pathogenicity of this parasite observed by various workers is given in a table by one of us in a previous paper, and it will be seen that the virulence of the different strains varies enormously. This fact is even more clearly brought out by a study of *T. congolense* (*T. dimorphon*), which we isolated from a horse naturally infected with this parasite and *T. vivax.* The early inoculations of the *T. congolense* from this horse as a rule failed to infect rats, rabbits and guinea-pigs. This applied also to the sub-inoculations for several passages; as the strain was passed from rat to rat it gradually became more virulent, until after passage through a series of about twenty animals it reached a state of equilibrium, killing them regularly in eight or nine days. Thus the pathogenicity of the trypanosome which at first resembled that of *T. nanum* was altered artificially, so that it became acutely virulent for rats and other laboratory animals. On the other hand, the virulence for goats of the *T. vivax* has not altered during a period of two and a half years, in which the strain has been passed through a series of forty of these animals.

It is obvious, therefore, that great caution must be exercised in expressing an opinion as to what is the species of a given trypanosome from the length of time which it takes to kill laboratory animals. This is so at least in the case of trypanosomes maintained by artificial passage through experimental animals. Probably one is on safer ground in the tropics, where the animals can be infected naturally by the bites of the insect host, as undoubtedly one of the results of the cycle of the parasite in the invertebrate host is to preserve the trypanosome in what might be termed its normal state.

It has been stated that certain trypanosomes, e.g., *T. vivax*, *T. nanum* and *T. theileri*, etc., do not infect small laboratory animals. In the past this qualitative reaction has sufficed to distinguish between *T. congolense* and *T. nanum*. More recent work on these parasites, however, has shown that this difference in pathogenicity is apparent only and that probably *T. nanum* and *T. congolense* are identical.

IV. SYMPTOMATOLOGY

In our experience, in so far as small laboratory animals and goats, donkeys and horses are concerned, it is impossible to state from the symptoms exhibited with which species of trypanosome the animals are infected. Pathological appearances, such as subcutaneous swellings, loss of hair, blepharitis, interstitial keratitis, oedema and ulceration of the genitals and mucous membrane are produced equally by many different species of trypanosomes. We have noted the swelling of the face in goats infected with *T. gambiense*, so that this cannot be considered as of any value as a diagnostic point distinguishing it from *T. rhodesiense*. We are inclined to think the plaques described in horses infected with *T. equiperdum* are characteristic of infection with this trypanosome, as it is only in animals infected with this strain that we have observed them. To sum up, quantitative reactions and symptomatology are of little importance as an aid to diagnosis, whereas qualitative reactions are perhaps more valuable.

V. SERUM DIAGNOSIS

As regards agglutinins, cytolytic sera, phagocytosis, phenomenon of attachment, and complement deviation, it is now pretty generally admitted that these are not specific, and are hence of no diagnostic value.

Great stress is laid by Laveran and the French School generally on the value of immunisation and cross immunisation. We have, unfortunately, no experience of these, and are hence unable to express an opinion as to their value, but might point out in this connection that of the many thousands of susceptible animals, including monkeys, dogs, goats, donkeys and horses, inoculated experimentally with pathogenic trypanosomes at Runcorn, two animals only (rabbits) have apparently resisted infection—one was inoculated repeatedly with *T. equiperdum* and the other with *T. gambiense*.

INVERTEBRATE HOST

Since Kleine's classical researches in 1909 on the development of *T. brucei* and *T. gambiense* in *G. palpalis*, much work has been done on the cycle of the various pathogenic trypanosomes in the invertebrate host. The information afforded by these investigations suggests that the manner in which the parasites affect the tsetse (or other invertebrate host) may be of considerable value as an aid to the differentiation of species.

1. *Specific host*. It was originally thought that a given trypanosome could be transmitted by one particular tsetse only. This is now known not to be so, *T. gambiense*, for example, can complete its development in both *G. palpalis* and *G. morsitans* *T. vivax* has been shown to be transmitted by *G. palpalis* and *G. morsitans*, whilst Roubaud and his colleagues found that *T. pecaudi* developed in *G. longipalpis* and *G. tachinoides*.

It is obvious, therefore, that if a certain trypanosome has been shown to be transmitted by one kind of tsetse and another to be transmitted by a second kind, this fact of itself is no sufficient ground for considering them different species. As a matter of fact, it seems within the limits of probability that all varieties of tsetse are capable of transmitting most, if not all, of the African pathogenic trypanosomes.

2. *Anatomical parts of the fly affected.* Whilst the fact that a certain species of tsetse transmits a given trypanosome is of no diagnostic assistance as far as the trypanosome is concerned, yet the manner in which the trypanosome affects the fly is possibly of considerable value. This subject has been dealt with by Roubaud and Duke. To make use of Duke's expression, 'before a trypanosome completes its development in the tsetse it takes up what may be termed its *anterior station.*' It is the locality of this *anterior station* which is of assistance in differentiating trypanosome species. By this means the African trypanosomes may be divided into the following three groups:—(i) Those in which the development of the parasite is limited to the proboscis, *T. vivax* (*T. cazalboui*), *T. uniforme*, *T. caprae.* (ii) Those in which the first portion of the developmental cycle takes place in the gut and from thence the infection spreads forwards and takes up its *anterior station* in the proboscis, *T. nanum*, *T. congolense*, *T. simiae*, *T. pecaudi.* (iii) Those in which the first portion of the cycle takes place in the gut and the final portion in the salivary glands, e.g., *T. gambiense*, *T. rhodesiense* and a polymorphic trypanosome described by Duke in Uganda.

3. *Period of incubation of trypanosomes in Glossina.* The work of Kinghorn and Yorke has shown that this depends to a great extent upon the temperature to which the fly is subjected, and therefore it is of no great value as a diagnostic aid.

4. *Morphological characters of the trypanosomes in the tsetse.* All trypanosomes, so far as is known at present, undergo marked morphological changes during their development in the invertebrate host. Trypanosomes morphologically distinct in the mammalian blood appear identical in the intestine of the insect. Again, the predominant types of such parasites as *T. gambiense* and *T. rhodesiense* found in the gut are quite distinct from those found

Group	Sub-Group		Trypanosome	Date and designators	Synonyms	Motility
I	A	1	*T. brucei*	1899 Plimmer & Bradford	...	...
		2	*T. evansi*	1885 Steel	*T. soudanense* (1907) *T. venezuelense* (1910)	... ...
		3	*T. hippicum*	1910 Darling	...	...
	B	4	*T. equiperdum*	1901 Doflein	...	...
	C	5	*T. equinum*	1901 Voges	...	...
	D	6	*T. vivax*	1905 Ziemann	*T. cazalboui* (1906)	**Very rapid, transla**
		7	*T. caprae*	1910 Kleine	...	„ „
		8	*T. uniforme*	1911 Bruce	...	...
	E	9	*T. transvaaliense*	1903 Laveran	...	...
		10	*T. theileri*	1902 Laveran	...	...
	F	11	*T. ingens*	1909 Bruce	...	...
		12	*T. tragelaphi*	1913 Kinghorn & Yorke	...	...
	G	13	*T. lewisi*	1881 Kent	...	**Very rapid, transla**
	H	14	*T. cruzi*	1909 Chagas	...	Tumbling movement
II	I	15	*T. congolense*	1904 Broden	*T. dimorphon* (1904) *T. pecorum* (1910) *T. confusum* (1909)	...
		16	*T. nanum*	1905 Laveran	...	...
	J	17	*T. montgomeryi*	1909 Laveran	...	...
	K	18	*T. simiae*	1912 Bruce	*T. ignotum* (1912)	...
III	L	19	*T. gambiense*	1902 Dutton	...	...
		20	*T. multiforme*	1912 Kinghorn & Yorke	...	...
		21	*T. nigeriense*	1913 Macfie	...	...
	M	22	*T. pecaudi*	1907 Laveran	...	...
		23	*T. rhodesiense*	1910 Stephens & Fantham	...	...
		24	*T. ugandae*	1913 Stephens & Blacklock	*T. brucei* (Uganda) (1909)	...
		25	*T. equi*	1913 Blacklock & Yorke	...	...

in the salivary glands. Until more is known of this subject, the morphological appearances of the trypanosomes in the tsetse can be of little use as an aid to differentiation of species.

In the following table we have attempted to classify the more important mammalian trypanosomes, making use of those methods of differentiation which are mentioned in this paper as being of value. We do not claim that a complete account is given of the pathogenic trypanosomes, but those points are emphasised which may be considered as characteristic, and hence of practical value for the differentiation of species.

DESCRIPTION OF PLATE I

Films fixed in alcohol and stained with Giemsa. The figures were drawn with the aid of a camera lucida at a magnification of 2,000 diameters.

Fig. 1. Represents the trypanosomes comprising sub-group A, viz., *T. brucei*, *T. evansi* and *T. hippicum*.

Fig. 2. *T. equiperdum*.

Fig. 3. *T. equinum*.

Fig. 4. *T. vivax*.

Fig. 5. *T. transvaaliense*. (After Laveran and Mesnil.)

Fig. 6. *T. theileri*. (After Laveran and Mesnil.)

Fig. 7. *T. tragelaphi*.

Fig. 8. *T. lewisi*.

Fig. 9. *T. cruzi*.

Fig. 10. *T. congolense* or *T. nanum*.

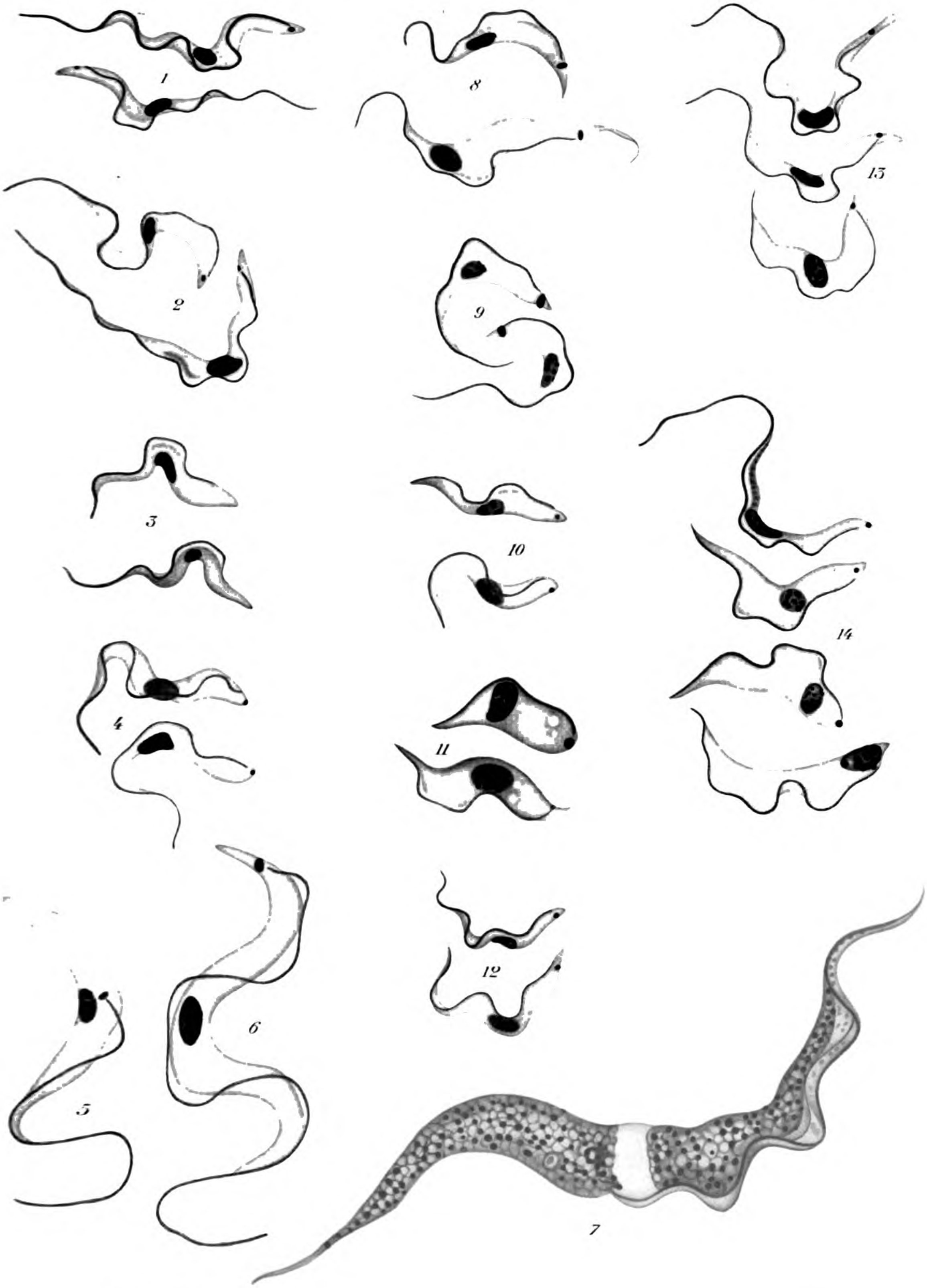
Fig. 11. *T. montgomeryi*.

Fig. 12. *T. simiae* (*T. ignotum*).

Fig. 13. Represents the trypanosomes comprising sub-group L, viz., *T. gambiense*, *T. multiforme* and *T. nigeriense*.

Fig. 14. Represents the trypanosomes comprising sub-group M, viz., *T. pecaudi*, *T. rhodesiense*, *T. ugandae* and *T. equi*.

PLATE I



A. M. Brookfield, del.

[illegible]

BRONCHIAL SPIROCHAETOSIS IN UGANDA, WITH PNEUMONIC SYMPTOMS

BY

J. A. TAYLOR, M.B. (EDIN.), D.T.M. (L'POOL)
MEDICAL OFFICER, UGANDA PROTECTORATE

(*Received for publication* 18 *November*, 1913)

Several cases of pneumonia of short duration and presenting some unusual features have come under my care recently at Entebbe Civil Hospital, and, previously, elsewhere in Uganda. As all the cases occurred among natives, it seemed probable that defective history might account for the apparently short duration, but the definite and constant symptoms and the finding of a large number of spirochaetes in the sputum of two recent cases make it possible that the condition may be caused by the extension of these parasites along the air passages. The history and course of the cases that have come under my care are as follows:—

For a few days the patient has a cold, but otherwise feels well and is doing his ordinary work. More or less suddenly a headache develops with some photophobia, and complaint is made of pains in the limbs and various parts of the body, but especially about the chest. The patient feels hot and begins to expectorate sputum streaked with blood. He passes a restless night, the pain and constant coughing preventing sleep, and in the morning appears to be in such a serious condition that his friends think he is about to die, and bring him to the hospital. On admission the case appears, at first sight, to be one of typical pneumonia of several days duration, and it is difficult to believe that the patient felt well and was doing his ordinary work on the previous day. The temperature varies between 102° and 104°, pulse 120 to 140, respirations 25 to 40. The cough is troublesome and a quantity of typical, tenacious, rusty-coloured sputum is expectorated. The patient complains of headache and pain in the chest, chiefly behind the sternum. On examination of the chest, sometimes nothing definite can be determined, but more often fine crepitations are heard posteriorly over a small and well defined area in the inferior lobe. In the cases I have seen, only one (the right) lung has shown any signs of being affected. Usually neither dulness nor bronchial breathing can be made out with certainty. The patient remains in the same condition from a few to forty-eight hours, when the temperature suddenly falls to sub-normal, and he feels comparatively well and makes an uninterrupted recovery without any relapse. The sputum remains rusty for perhaps thirty-six hours after the fall of temperature, then gradually becomes more like that of an ordinary cold and continues for a week or ten days in diminishing quantities. Several medical men in Uganda inform me that they have had almost exactly similar cases, but consider any peculiarities might be accounted for by the indefinite history, and also the sputum has not been examined microscopically.

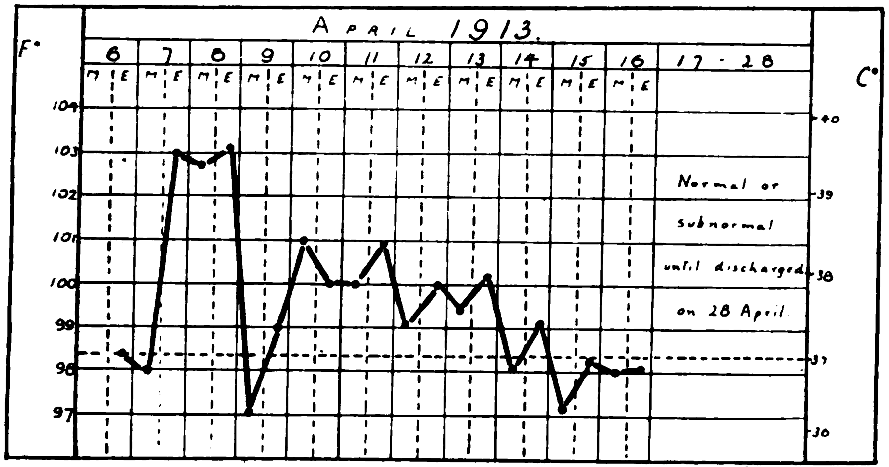
In the two recent cases recorded above in which spirochaetes were found in the sputum, a trustworthy history was, however, obtainable. Both patients had been working for the same employer and sleeping in the same room. On April 6th, 1913, some dead rats were found near their quarters, and as a suspected case of plague had occurred in Entebbe a few days previously, the two servants, with other natives, were kept under observation pending a thorough examination of the rats.*

On April 6th and the following morning, beyond some with colds, all the natives under observation appeared well, had no temperature and made no complaints of feeling ill. The further history of the two cases is as follows:—

Case 1. States he had a cold for a few days but otherwise felt well until the afternoon of the 7th April. He then complained of headache and pain in the chest and was removed to hospital and isolated. From normal in the morning his temperature rose to 103° in the evening, and the cough became more painful and troublesome. The eyes were slightly congested and there was some photophobia. During the night the sputum, at first containing streaks of blood, became rusty coloured, and on microscopical examination it was found to contain a large number of spirochaetes, as many as five being seen in one field under the 1/12 oil immersion lens. Fine crepitations could be heard at the base of the right lung, and there appeared to be slight dulness on percussion over the same area. The temperature remained at 103° for 24 hours and then during the night of the 8th April fell to sub-normal. The sputum remained rusty until the morning of the 10th and spirochaetes were found in it in decreasing numbers until the 12th. After the 12th, although expectoration continued for several days, no spirochaetes could be found.

---

* No evidence of plague was found. The rats were probably poisoned but the exact cause of their death was not determined.

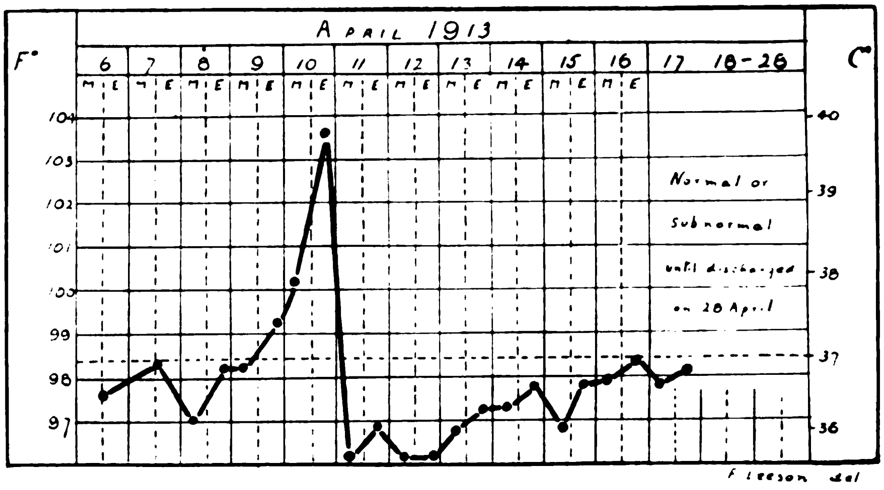


Fleeson del

Temperature Chart. Case I

The rise of temperature on the 8th and following day was, probably, chiefly due to the effects of a prophylactic plague injection.

Case 2. This patient slept in the same room as the previous one, and also stated that he had had a cold for some days. On April 6th, 7th and 8th, he felt well and his temperature was normal. On the 9th the sputum contained streaks of blood, and the temperature began to rise and reached 103·8° the following evening. Rusty-coloured sputum was expectorated during April 10th, and spirochaetes were found on both the 9th and 10th, but in less numbers than in the previous case. Fine crepitations could be heard on the right side over a small area, about the middle of the inferior lobe. Neither dulness nor bronchial breathing could be detected. During the night of April 10th the temperature fell to 96°, and afterwards, although expectoration continued for several days, neither spirochaetes nor blood corpuscles were again found in the sputum.



Temperature Chart. Case II

In this case the patient received no prophylactic plague inoculation, and the temperature remained normal until his discharge from hospital.

In both cases after the fall of temperature, the patients stated they felt quite well and wished to leave the hospital the following day. The possibility, however, of a relapse in any condition due to spirochaetes was entertained, and they were therefore detained in hospital until 28th April, but no relapse occurred. The only treatment carried out in both cases was the administration of a dose of salts on admission, and a mild expectorant mixture every three hours. The blood was carefully examined on numerous occasions for spirochaetes but with negative results. The spirochaetes were found in the sputum after staining with ordinary strength Leishman for two minutes, and for ten minutes with diluted stain. The parasites were obviously much shorter and less coiled than the common blood spirochaetes of African Relapsing Fever. Dr. Fantham kindly examined a slide sent to him in May, 1913, and reports that he found spirochaetes of two forms, viz.:—

(1) Short, slightly thicker and more darkly staining.

(2) Longer, delicate and pale staining forms.

He was unable at the time to identify them with certainty, but considered them of the gut or bronchial variety. He suggested that they were *S. bronchialis*. He states that the blood spirochaetes are much more readily stained, and that for the gut and bronchial varieties thionin is the quickest stain, and hot Giemsa better than ordinary Giemsa. Haematoxylin is useful.

Taking all the facts into consideration, there certainly appears to be some evidence to point to the probability of the spirochaetes found in the two cases described having been the cause of the disease.

References to literature, kindly given me by Dr. Fantham, show that Castellani in Ceylon, Branch in the West Indies and Jackson in the Philippine Islands have shown that spirochaetes have been the cause of bronchitis, and I understand, more recently, Chalmers has found similar cases in the Sudan.

There seems, therefore, no reason why spirochaetes should not, by further extension into the lungs, excite or cause a mild or even severe type of pneumonia. The chief difficulty of proof as to causation lies in the fact that spirochaetes (only in comparatively small numbers so far as I can determine) can often be found in the mouth, and expectoration without any obvious symptoms. Special precautions, however, were taken in the two cases described to prevent contamination from the mouth and pharynx. The mouth was well rinsed out with water and the patient expectorated first into one receptacle and then into another. The first and last particles of sputum brought up at one time could thus be examined and compared, but showed no obvious difference in the number of spirochaetes.

The total disappearance of the spirochaetes from the sputum within a few days, and the fact that the decrease in their numbers synchronized with the abatement in symptoms, are also significant.

Stomatitis, pharyngitis and respiratory diseases are very common among the natives of Uganda, and it is possible that some of these conditions may be due to spirochaetes, or, perhaps more probably, the lowered resistance and suitable nidus thus provided, may allow parasites usually harmless to increase in numbers and virulence to such an extent that they become of pathological importance.

ADDENDUM, *December 24th*, 1913.—Since writing the above, spirochaetes have been found in smears of sputum from three cases of natives suffering from acute bronchitis, of an Indian suffering from coughs and colds for six months, and of a native suffering from what was at first diagnosed as pneumonia. A case of bronchial spirochaetosis has also been found recently among Europeans in Entebbe. These records may be of interest. They are mentioned here as probably it will not be possible for me to follow up the present cases.

REFERENCES

CASTELLANI. Lancet, May 19, 1906.

CASTELLANI and CHALMERS. Manual of Tropical Medicine, 2nd edition, 1913.

CHALMERS and O'FARRELL. Journ. Trop. Med. and Hyg., Nov. 1, 1913.

BRANCH. British Medical Journal, 1907.

JACKSON. Philippine Journal of Science, 1908.

WATERS. Transactions Society of Tropical Medicine, 1909.

ATTEMPTS TO FIND DISEASE GERMS IN THE EUROPEAN BED BUG *(CIMEX LECTULARIUS)* AFTER FEEDING EXPERIMENTS IN VARIOUS DISEASES: LEPROSY, LYMPHADENOMA, CARCINOMA, ETC.

BY

DAVID THOMSON, M.B., CH.B. (EDIN.), D.P.H. (CANTAB.)
GROCERS' SCHOLAR

(*Received for publication* 1 *October*, 1913)

CONTENTS

		PAGE
I.	INTRODUCTION	19
II.	ORGANISMS NORMALLY FOUND IN THE BED BUG	21
III.	THE FEEDING OF BED BUGS ON PATIENTS	23
IV.	RESULTS OF THE FEEDING EXPERIMENTS :—	
	(*a*) Leprosy	24
	(*b*) Lymphadenoma (Hodgkin's Disease)	26
	(*c*) Spleno-Medullary Leukaemia	26
	(*d*) Chronic Lymphatic Leukaemia	26
	(*e*) Sarcoma	27
	(*f*) Carcinoma	27
	(*g*) Malaria	27
V.	SUMMARY AND CONCLUSIONS	27
	REFERENCES	28

I. INTRODUCTION

In recent years, the important part played by blood-sucking insects in the transmission of disease has been brought strongly into evidence. Mosquitos carry the pathogenic agents of malaria, yellow fever and filariasis. Tsetse-flies transmit various forms of sleeping sickness. Bugs and fleas are able to transmit plague germs, Manning (1912), whilst the former may be responsible for the transmission of kala-azar, Patton (1907, 1912), and possibly of leprosy, Long (1911). By feeding experiments Patton (1911) and Wenyon (1912) were able to get some development of Leishmania and allied flagellates in bed bugs, fleas and mosquitos. Dewèvre

(1892) found tubercle bacilli in bed bugs living in the bed of a tuberculous host. These diseases, the latter excepted, are confined chiefly to the tropics, but it is not known whether the blood-sucking insects which occur in temperate climates may not transmit some of the diseases which occur in the temperate zone. Bugs, fleas and lice are all very common in the civilised countries of temperate climes, in spite of the great advances made in sanitation and personal cleanliness. It appears, therefore, that those engaged in research on non-tropical diseases, many of which are still shrouded in mystery, have neglected this department of research, which is being so thoroughly investigated by those engaged in the study of diseases peculiar to the tropics. No one appears to have attempted to investigate such diseases as lymphadenoma and the various leukaemias, from this point of view. These are diseases in which the blood is affected, and, moreover, they resemble certain of the tropical diseases mentioned, in the enlargement of the spleen and lymphatic glands. Recently I was much impressed by the remarkable resemblance between a case of advanced lymphadenoma and kala-azar. This case was a young child five years of age. She had enlarged glands and a very large spleen, extending below the umbilicus. There was extreme emaciation, anaemia and lassitude, and a few days before death she developed an ulcer on her cheek, which rapidly perforated, forming a kind of cancrum oris, all of which features are also characteristic of kala-azar. This induced me to commence feeding experiments with bed bugs on this case of lymphadenoma and on other obscure blood diseases, which constantly come into hospital in this country. Crithidia have been found in fleas, Porter (1911), and it has been recently shown by Fantham (1912) that 1 per cent. or more of body lice in England contain Herpetomonad flagellates, the cysts of which are found in their faeces. Fantham did not succeed in infecting either himself or animals with these parasites, but their presence in these insects is suggestive. No one appears to have observed protozoal flagellates in the bed bug, but they harbour certain organisms which I shall describe below. It appears to me that a considerable research should be conducted with blood-sucking insects regarding the obscure diseases occurring in temperate climates. Although it has not been ascertained whether or not these obscure diseases are due to organisms, yet so long as they are obscure, we must consider the possibility of organismal causes. Yellow fever was found to be transmitted by *Stegomyia fasciata*, although no organism could be discovered in the blood of the patients, so that the inability to demonstrate organisms in an obscure disease should not debar an investigation with blood-sucking insects. It is always possible that a phase in the development of a pathogenic organism might appear in some of these insects fed on patients suffering from disease, a phase more easily seen through the microscope than in the blood of the patient. In malaria some of the parasitic phases in the mosquito are much more gross, so to speak, than the phases in the blood of the human host. Finally, the blood sucked by the insect into its gut becomes digested and changed, and may be considered as a kind of culture medium in which an obscure organism may develop. J. G. Thomson and Sinton (1911) have shown that the growth and morphological changes in human trypanosomes in culture media, are exactly similar to those occurring in the gut of the tsetse-fly which has sucked blood infected with these organisms. I have ventured to make these remarks as a plea for further research with insects regarding the obscure diseases which occur in temperate climates, and which have so far baffled all other methods of scientific investigation.

II. ORGANISMS NORMALLY FOUND IN THE BED BUG

The number of control bed bugs examined in this research was 184. Of these, 107 were examined for acid fast bacilli, by staining with the Ziehl-Neelsen stain. The remaining 77 were stained by the Giemsa method. Eight of these latter were fed on a normal person. The remaining 69 were examined as soon as they had been obtained from their natural haunts. I speak of these as controls. The majority of them were adult bugs and had undoubtedly fed on human beings, but whether any of these human beings had been suffering from disease it is impossible to say. The only method of obtaining perfect controls would be to hatch out the bugs from the eggs and rear them to the adult stage, feeding them at times on a normal person. I have not done this. However, I will now describe the various organisms which I have found in these so-called controls.

(*a*) *Bacterial organisms*: Bacilli were found in about 25 per cent. of the bugs. Usually these bacilli were scanty, but occasionally I have found them in masses, in the intestinal contents, and in the lining cells of the gut. They were, as a rule, long, slender bacilli. Stout bacilli of the coli type were practically never found, and I have seldom been able to detect cocci. I was unable to detect any acid-fast bacilli in the 107 controls stained by the Ziehl-Neelsen method.

(*b*) *Yeasts and Moulds*. These were more commonly found in the gut than bacteria. Sometimes a mycelium was found sprouting from the spores. On one occasion I transferred the intestinal contents of a bed bug as aseptically as possible into a blood agar tube. The only growth obtained from this was an abundant white mould with numerous spores and mycelium. Bacteria were seldom found in the faeces of these bugs, but on some occasions I have found fungal spores, and if the faeces are kept for some time in a moist atmosphere, a copious mouldy growth, as a rule, sprouts from them.

(*c*) *Protozoal organisms*. I have been unable to find any definite protozoa in the total 455 bugs examined, and never any flagellates such as have been found in fleas and lice.

(*d*) *Other bodies, possibly organismal*:

(1) Indefinite bacilliform bodies occurred in about 10 per cent. of the bugs. These bodies occurred in masses in the protoplasm of the gut cells and were also found in the intestinal contents. They appeared to me to be a species of organism attacking the cells of the intestine.

(2) 'Chlamydozoon-like bodies': These occurred in about 25 per cent. of the controls. They were found chiefly in the cells of the oesophagus, less commonly in the cells of the mid and hind gut, and only occasionally did they occur free in the intestinal contents.

I have called them 'Chlamydozoon-like bodies' because they resemble the bodies to which Prowazek has applied the name of Chlamydozoa, and which are believed to be organismal. Similar bodies have been described by Leishman (1912). I am in considerable doubt as to whether these similar bodies found in oesophageal cells of the bug are organismal or whether they are of the nature of cell secretions. These bodies vary in appearance as follows: —

(1) A minute red staining point (after using Giemsa), almost invisible, within a clear circular area or vacuole.

(2) A minute red staining point with a clear halo around it. This clear halo, in turn, is bounded by a red staining ring.

(3) A red granule without any halo around it.

(4) A small spherical red body, paler in the centre than at the periphery, and without any surrounding halo.

These bodies, as a rule, stain red in colour, but where the tissue is thick, so that the stain is too dark, they show up as dark-blue bodies, varying in size from a minute granule up to 2μ and even 3μ in diameter. They frequently occur in pairs partially fused together, as if they were dividing into two.

III. THE FEEDING OF BED BUGS ON PATIENTS

This is very easily done. I have found the following procedure very suitable. Procure some glass tabloid phials with their metal caps.* The metal caps which I used were about ¾ inch in diameter and about ½ inch deep. Transfer about six bugs into one of these metal capsules. The bugs are easily transferred from one vessel to another, by using a wire with a loop at one end. With this turn the bug on its back and then place the loop among its legs. It will cling to the loop and can then be shaken off into the metal cap. Enclose the bugs in this metal capsule by stretching a single layer of surgical gauze, such as is used for dressings, over the mouth of the capsule. This is secured in position by a piece of plaster wrapped round the side of the capsule. Shake the bugs from the bottom of the capsule on to the gauze and place it face downwards on the patient's forearm. Secure it in that position against the patient's skin with a piece of plaster. If this is left undisturbed for 5 to 10 minutes, it is almost certain that the bugs will be found well engorged with blood. They bite very readily through the gauze, provided it is stretched tightly across the mouth of the capsule. In this fashion I have frequently fed 12 bugs in 5 minutes.

---

* I used the phials which are frequently sent out by the Wulfing Co. with samples of formamint tabloids.

The patient does not feel much, and, in the majority of cases, the bugs scarcely leave any visible mark. On only one of my patients they produced slight blisters. On several occasions I was able to feed these insects without waking the patient, especially in the case of young children. After feeding the bugs, they should be transferred to the glass phial to which the capsule belongs. A piece of cardboard should be placed in the phial to act as a foothold for them, and they deposit their excreta and eggs on this cardboard. I fed them only once, and killed and examined them at intervals varying from 4 to 19 days after feeding. A large number should be fed on several cases of a given disease, before coming to any conclusion. It has been found, for example, that only about 5 per cent. of Anophelines fed on a malarial patient become infective, and there are many cases of malaria which would never infect anophelines, as they do not always contain gametes. Similarly, only about one in twenty of tsetse-flies fed on cases of sleeping sickness become infective. Again, conclusions cannot be drawn from the microscopical examination of the bugs alone, for although no abnormal organism can be found in them after feeding on a given diseased patient, yet they may be carrying some invisible virus, as in the case of yellow fever. To complete the experiment, therefore, insects which have fed on a given disease should be allowed to bite other men or animals at varying periods after their feed.

The feeding experiments which I am about to describe have been very incomplete, as I have not been able to employ sufficient numbers and also because I have not allowed the bugs to bite other men or animals. Hence, although my results have been so far negative, there is no room for dogmatic statements that they do not carry any of the diseases on which I have experimented.

IV. RESULTS OF THE FEEDING EXPERIMENTS ON DISEASED PATIENTS

(*a*) *Nodular Leprosy*. Twenty-one bed bugs, *Cimex lectularius*, caught in Liverpool, were fed directly on the nodules of a patient in the Royal Southern Hospital. They were killed and examined for acid-fast bacilli at periods varying from one to ten days after feeding. Two bugs were examined two months after feeding.

Result.—No acid-fast bacilli could be found in the salivary glands, intestines or faeces. Twenty-one controls were examined in a similar fashion and no acid-fast bacilli were found.

I made further investigations in Panama and also in Trinidad. Eighty-six bed bugs (*Cimex rotundatus*) were caught in Ancon Hospital, Panama, and examined as controls, but no acid-fast bacilli could be detected in them (Ziehl-Neelsen stain used). Eighty-four bed b ugs (*Cimex rotundatus*) caught in Ancon Hospital were fed on seven lepers at Paloseco leper asylum, Panama. In six of the lepers chosen the disease was advancing rapidly, and one exhibited a recent crop of small nodules. The bugs were fed directly on the nodules, but not on open sores. The seventh case exhibited a scaly condition of the skin. The bugs were allowed to feed on the patients for a period of half an hour and were engorged with blood after the application. These bugs were killed and examined for acid-fast bacilli at periods varying from one to fifteen days after the feeding. No acid-fast bacilli could be detected in their alimentary tract or faeces, nor in their salivary glands.

Seventeen bed bugs caught in Paloseco leper asylum were similarly examined and no acid-fast bacilli were detected. These latter bed bugs were caught on the mattresses of the beds of lepers. They contained blood and had presumably fed frequently on leper patients.

Eighteen bed bugs (species not determined) were caught on the mattresses of the beds of lepers in the Trinidad leper asylum. The examination of these insects for acid-fast bacilli was also negative.

In a total of 107 control bugs no acid-fast bacilli were found. This agrees with the investigations of Long (1911) and Sandes (1911).

In a total of 105 bed bugs fed on lepers and 35 caught on the mattresses of leper patients, I was unable to detect acid-fast bacilli. In this respect I have been unable to confirm the results of Long and Sandes.* It seems to me, therefore, that bed bugs are not so liable to ingest lepra bacilli from patients as has been maintained. However, it is conceivable that they might ingest these bacilli by feeding on very acute cases of leprosy, as in such cases the bacilli have been found occasionally in the circulating blood.

---

* Sandes (1911) and Long (1911) found acid-fast bacilli in twenty-six per cent. of the bed bugs which they fed on leper patients.

Skelton and Parham (1913) were unable to find acid-fast bacilli in 175 bed bugs caught in the beds of lepers in Zanzibar.

Vide also the experiments of Paldrock; Smith, Lynch and Rivas (1913).

(*b*) *Lymphadenoma* (*Hodgkin's Disease*). Five bed bugs were fed on a very advanced case of this disease three days before the death of the patient. All of these developed in 8 days a greyish, mouldy growth over their bodies, and microscopic examination showed that they were infected with a mycelium and spores. Twenty-two bugs were fed on an early case of this disease. These were examined at varying periods up to 13 days after feeding. They did not show anything different from what was found in the controls, except that 63 per cent. of them contained 'Chlamydozoon-like bodies.' The peculiar condition of the first five bugs may have been a coincidence, as occasionally mould-like spores and even mycelial threads have been found in the controls, but always very scantily.

(*c*) *Spleno-Medullary Leukaemia.* Forty bed bugs were fed on one typical case of this disease and examined at varying intervals of from 4 to 19 days afterwards. All of them showed one persistent abnormality from the control bugs. This was the constant presence of Charcot-Leyden crystals in their intestinal contents and even in their faeces. These crystals were practically always abundant. They were colourless, pointed at both ends and varied in size from about 7μ to 40μ in length. They occurred singly and in small groups, and were, apparently, very brittle and easily fractured. They stained a faint greenish colour with the Giemsa stain.

Fifty per cent. of these bugs contained numerous 'Chlamydozoon-like bodies,' principally in the cells of the oesophagus.

(*d*) *Chronic Lymphatic Leukaemia.* A very typical case was used, which presented a leucocyte count of 800,000 per c.mm., of which 97 per cent. were small or medium sized lymphocytes. None of the 22 bed bugs fed on this case showed any apparent abnormality, except that the majority showed very large numbers of pale blue bodies in the intestinal contents. These were undoubtedly the remains of the ingested lymphocytes. Sixty per cent. of them showed numerous 'Chlamydozoon-like bodies' in the cells of the oesophagus. Not one of them showed Charcot-Leyden crystals. These bugs were killed at intervals of from 4 to 13 days after feeding.

(*e*) *Sarcoma.* Twenty-eight bugs were fed on a patient suffering from sarcoma of the ileum. These were examined at intervals varying from 5 to 16 days after feeding, but they revealed nothing abnormal. Forty-three per cent. showed 'Chlamydozoon-like bodies' in the oesophageal cells.

(*f*) *Carcinoma.* Six bugs were fed on an advanced case of cancer of the bowel, but nothing abnormal was found in them.

(*g*) *Malaria.* Eight bugs were fed on a case of malignant tertian malaria, with crescents. They revealed no development of the malarial parasite within them.

I am much indebted to Dr. Macalister of the Royal Southern Hospital, Liverpool, for his kindness in allowing me to perform these insect feeding experiments on several of his patients.

V. SUMMARY AND CONCLUSIONS

(1) Four hundred and fifty-five bed bugs were employed in this research, of these, one hundred and eighty-four were used as controls.

(2) Insufficient research has been carried on with regard to the possibility of the transmission of disease in temperate climates, by blood-sucking insects occurring in these climates.

(3) Protozoal parasites do not appear to exist normally in the European bed bug.

(4) No acid-fast bacilli were found in 105 bed bugs fed on lepers, nor in 35 bed bugs caught on the bed mattresses of leper patients.

(5) In the few experiments conducted, nothing abnormal was found in bugs fed on cases of lymphadenoma, chronic lymphatic leukaemia, sarcoma, carcinoma and malaria.

(6) Forty bed bugs fed on a case of spleno-medullary leukaemia all developed numerous Charcot-Leyden crystals in their intestines.

REFERENCES

Dewèvre, — (1892). Medical Record.

Fantham, H. B. (1912). *Herpetomonas pediculi*, nov. spec., parasitic in the alimentary tract of *Pediculus vestimenti*, the human body louse. Proc. Roy. Soc., B, Vol. LXXXIV, pp. 505-517. One plate.

Leishman, Sir W. B. (1912). Cell-inclusions in the Blood of a Case of Blackwater Fever. Jour. Roy. Army Med. Corps, Vol. XVIII, pp. 493-504.

Long, E. C. (1911). A Note on the Transmission of Leprosy. Brit. Med. Jour., Sept. 2nd, 1911.

Manning, J. v. V. (1912). Bed Bugs and Bubonic Plague. Med. Record, July 27th, Vol. LXXXII, No. 4, pp. 148-150.

Paldrock, A. (1913). Wanzen und Schaben als Verbreiten des Lepraerregers. Dermatol. Centralbl. December. Vol. VII, No. 3, pp. 66-71.

Patton, W. S. (1912). The Development of the Parasite of Indian Kala-azar (*Herpetomonas donovani*, Laveran and Mesnil) in *Cimex rotundatus*, Sign, and in *Cimex lectularius*, Linn., with some observations on the behaviour of the parasite in *Conorrhinus rubrofasciatus*, de Geer. Sci. Mems. by Officers of the Med. and Sanit. Depts. Govt. of India, Calcutta, New Ser., No. 53, 38 pp. and one plate.

——— (1907). See also Sci. Mem. Govt. India, No. 31.

Porter, Annie (1911). The Structure and Life-History of *Crithidia pulicis*, n.sp., parasitic in the alimentary tract of the human flea, *Pulex irritans*. Parasitology, IV, pp. 237-254. One plate.

Sandes, T. L. (1911). The Mode of Transmission of Leprosy. Brit. Med. Jour., Sept. 2nd, p. 469.

Skelton, D. S. and Parham, J. G. (1913). Leprosy and the Bed Bug. Jour. Roy. Army Med. Corps. March. Vol. XX, No. 3, pp. 291-292.

Smith, Allen J., Lynch, K. M. and Rivas, Damaso (1913). The Transmissibility of the Lepra Bacillus by the Bed Bug. (*Cimex lectularius L.*). Amer. Jour. of Med. Sciences. November. Vol. CXLVI, No. 5, pp. 671-681.

Thomson, J. G. and Sinton, J. A. (1912). The Morphology of *Trypanosoma gambiense* and *Trypanosoma rhodesiense* in cultures; and a comparison with the Developmental forms described in *Glossina palpalis*. Ann. Trop. Med. and Parasitol., Vol. VI, pp. 331-356. Three plates.

Wenyon, C. M. (1912). Experiments on the Behaviour of Leishmania and allied Flagellates in Bugs and Fleas, with some Remarks on Previous Work. Jour. London School Trop. Med., Vol. II, pp. 13-26.

ON THE PATHOGENICITY OF THE TRYPANOSOME (*T. NIGERIENSE*) FROM A CASE OF SLEEPING SICKNESS FROM EKET, SOUTHERN NIGERIA

BY

J. W. SCOTT MACFIE, M.A., M.B., Ch.B.

(WEST AFRICAN MEDICAL STAFF)

(*Received for publication* 20 *December*, 1913)

When describing, in a former paper, certain morphological features of a trypanosome obtained from a case of sleeping sickness at Eket in Southern Nigeria,[1] I called attention to the fact that the parasite appeared to have a low degree of virulence when inoculated into the smaller laboratory animals, and added a short table embodying my results up to the time of writing. Since then I have continued the investigation of the animal reactions of this trypanosome, and the following is an account of the results obtained.

Considerable difficulty was experienced in procuring a sufficient supply of animals for experimental purposes, and for this reason it was impossible to carry out as large a number of inoculations as I should have wished. Unfortunately no white mice were available, and until recently I was unable to obtain white rats. For experimental purposes wild rats and mice are not satisfactory owing to the difficulty of handling them; and moreover, the majority of the rats caught near Lagos are infected with *T. lewisi*. I regret also that it was necessary to discontinue these experiments somewhat prematurely, owing to the fact that I was leaving Lagos.

The strain of trypanosome used in the following experiments was obtained from a guinea-pig inoculated by the late Dr. Foran with cerebro-spinal fluid from one of the cases of sleeping sickness under his care at the isolation camp near Eket. All the first inoculations were made with the blood of this animal; but after its death, which occurred on the 158th day, blood from two other infected guinea-pigs, and one infected monkey, was used.

All the inoculations, with the exception of two, were subcutaneous, and the quantity of infective blood employed was in each case small. The latter precaution seemed necessary, in view of the experiences of Beck,[2] who explains the uncertain effects of inoculations with larger volumes of the infective blood to the presence of anti-bodies which act as trypanocides. The blood was diluted with 1 % sodium citrate solution before use.

In most of the animals which became infected trypanosomes were scanty. In no case did their number approximate to that found, for example, in a guinea-pig infected with *T. brucci (ugandae)*. Parasites of the small type characteristic of the strain were seen, however, in all the animals in which the trypanosomes appeared in moderate numbers. None of the animals experimented with showed any symptoms, and in every case the disease was chronic. The appearance of the parasites in the peripheral blood of animals that were infected was inconstant; and on October 30th, the last day included for the purposes of this paper, all the examinations were negative.

RATS

Eight experiments were performed with rats; two with black rats, and six with white rats. Seven of the experiments were negative. In one animal a single trypanosome was found in the blood, on the 59th day. At a second examination on the same day, and on subsequent days, no parasites were found. Three of the rats died of intercurrent disease, but their tissues did not show any signs of trypanosomiasis. The other animals showed no sign of illness during the 48 to 69 days they were under observation.

TABLE I.—Rats inoculated with the *Nigerian strain.*

Animal	Inoculation	Day on which parasites were first seen in the blood	Day on which death occurred	Remarks
Black rat, No. 38	Subcutaneous from guinea-pig, No. 1	—	7th	Parasites never found in the blood. Died of intercurrent disease on the 7th day.
Black rat, No. 51	Subcutaneous from guinea-pig, No. 1	—	10th	Parasites never found in the blood. Died of intercurrent disease on the 10th day.
White rat, No. 75	Subcutaneous from guinea-pig, No. 23	59th	—	A single trypanosome seen once (on the 59th day). Animal alive and well after 69 days.
White rat, No. 76	"	—	—	Parasites never found in the blood. Animal alive and well after 69 days.
White rat, No. 77	"	—	—	" "
White rat, No. 117	Subcutaneous from guinea-pig, No. 22	—	14th	Parasites never found in the blood. Died of intercurrent disease on the 14th day.
White rat, No. 118	"	—	—	Parasites never found in the blood. Animal alive and well after 48 days.
White rat, No. 119	"	—	—	" "

MICE

Unfortunately, no tame mice were available, and it was, therefore, necessary to use the local wild mice (*Mus musculus*) for experimental purposes. These animals were difficult to handle, and were apt to be killed in catching them for examination. Seven experiments were carried out, three of which were positive, and four negative. In those animals that were infected the parasites were always either scanty or absent. One animal, which was under observation 107 days, did not show trypanosomes after the 48th day. The incubation period was 5 to 9 days. The disease was chronic. The duration was at least 107 days, but as no animal died of the disease, it is not possible to state any definite period. The infected animals presented no symptoms of illness.

TABLE II.—Mice inoculated with the *Nigerian strain.*

Animal	Inoculation	Day on which parasites were first seen in the blood	Day on which death occurred	Remarks
Mouse, No. 7	Subcutaneous from guinea-pig, No. 1	—	30th	Parasites never found in blood. Died on the 30th day. Tissues showed no signs of trypanosomiasis.
Mouse, No. 24	Subcutaneous from guinea-pig, No. 1	5th	51st	Parasites very scanty or absent. Accidentally killed on the 51st day. Animal showed no symptoms of illness.
Mouse, No. 26	Subcutaneous from guinea-pig, No. 1	7th	11th	Parasites scanty. Accidentally killed on the 11th day.
Mouse, No. 32	Subcutaneous from guinea-pig, No. 1	—	—	Parasites never found in the blood. Animal alive and well after 121 days.
Mouse, No. 35	Subcutaneous from guinea-pig, No. 1	—	—	" "
Mouse, No. 44	Subcutaneous from guinea-pig, No. 1	9th	—	Parasites rarely found, and then only in very small numbers. None seen for nearly two months. Animal alive and well after 107 days.
Mouse, No. 45	Subcutaneous from guinea-pig, No. 1	—	—	Parasites never found in the blood. Animal alive and well after 107 days.

GUINEA-PIGS

Twelve experiments were carried out with guinea-pigs. Three of the animals became infected, nine did not. The disease was very chronic, and the animals presented no symptoms of illness. The parasites were fairly constantly present in the blood, but usually in small numbers. They were never very numerous. The incubation period was 27 days. The duration was 158 days in the one animal that died of the disease.

TABLE III.—Guinea-pigs inoculated with the *Nigerian strain.*

Animal	Inoculation	Day on which parasites were first seen in the blood	Day on which death occurred	Remarks
Guinea-pig, No. 1	Subcutaneous from a case of sleeping sickness at Eket	(?)	158th	Parasites never present in large numbers, but generally fairly numerous.
Guinea-pig, No. 2	Subcutaneous from guinea-pig, No. 1	—	—	Parasites never found in the blood. Animal alive and well after 171 days.
Guinea-pig, No. 16	Intraperitoneal from guinea-pig, No. 1	—	16th	Parasites never found in the blood. Died of intercurrent disease. No evidences of trypanosomiasis in the tissues.
Guinea-pig, No. 17	Intraperitoneal from guinea-pig, No. 1	—	—	Parasites never found in the blood. Animal alive and well after 149 days.
Guinea-pig, No. 22	Subcutaneous from guinea-pig, No. 1	27th	—	Parasites often scanty or absent. Sent home to England on the 124th day. Animal at that time alive and well.
Guinea-pig, No. 23	Subcutaneous from guinea-pig, No. 1	27th	—	Parasites never very numerous and often either scanty or absent. Animal alive and apparently well after 139 days.
Guinea-pig, No. 120	Subcutaneous from guinea-pig, No. 22	—	—	Parasites never found in the blood. Animal alive and well after 48 days.
Guinea-pig, No. 121	Subcutaneous from guinea-pig, No. 22	—	—	Parasites never found in the blood. Animal alive and well after 48 days.
Guinea-pig, No. 122	”	—	—	” ”
Guinea-pig, No. 123	Subcutaneous from monkey, No. 21	—	39th	Parasites never found in the blood. Died on the 39th day of intercurrent disease. No signs of trypanosomiasis in the tissues.
Guinea-pig, No. 124	”	—	—	Parasites never found in the blood, Animal alive and well after 48 days.
Guinea-pig, No. 125	”	—	—	” ”

DOGS

Seven experiments were carried out with dogs. All proved refractory except one. This animal first showed trypanosomes on the 85th day. On subsequent days parasites were generally to be found in the blood in small numbers, sometimes, however, they appeared to be absent. The dog showed no symptoms of disease, and was still alive and in normal health on the 115th day.

Table IV.—Dogs inoculated with the *Nigerian strain.*

Animal	Inoculation	Day on which parasites were first seen in the blood	Day on which death occurred	Remarks
Dog, No. 13	Subcutaneous from guinea-pig, No. 1	—	93rd	Parasites never found in the blood. Animal died on the 93rd day of distemper and mange.
Dog, No. 14	Subcutaneous from guinea-pig, No. 1	—	—	Parasites never found in the blood. Animal alive and well after 149 days.
Dog, No. 36	Subcutaneous from guinea-pig, No. 1	85th	—	Parasites never numerous. Animal alive and apparently in good health after 115 days.
Dog, No. 106	Subcutaneous from guinea-pig, No. 23	—	—	Parasites never found in the blood. Animal alive and well after 55 days.
Dog, No. 107	”	—	—	” ”
Dog, No. 108	”	—	—	” ”
Dog, No. 109	”	—	—	” ”

MONKEYS

Only two experiments were performed owing to the difficulty of obtaining monkeys. The two animals were different, the one being a small red-haired monkey, and the other, also red-haired, a somewhat larger species. Both were inoculated with blood from the original guinea-pig (No. 1) obtained from Eket. The disease ran a chronic course, and at the time of writing both animals are alive, and appear to be in normal health. Trypanosomes first appeared in the peripheral blood on the 8th and 11th days after inoculation. No symptoms of disease have as yet manifested themselves, although it is now 118 and 137 days respectively since the injections were made. Throughout the course of the disease, so far as it has at present advanced, the trypanosomes have been scanty, and it has often been impossible to find them at all. In one

(No. 37) no trypanosomes were detected for over a month, namely, from the 53rd to the 90th day.

TABLE V.—Monkeys inoculated with the *Nigerian strain.*

Animal	Inoculation	Day on which parasites were first seen in the blood	Day on which death occurred	Remarks
Red-haired monkey, No. 21	Subcutaneous from guinea-pig, No. 1	11th	—	Parasites always scanty or absent. Animal alive and well after 137 days.
Small red-haired monkey, No. 37	,,	8th	—	Parasites always scanty or absent. Animal alive and well after 118 days.

GOATS

Two experiments were carried out with goats. One became infected. The disease was very chronic. Trypanosomes were only found in the blood on two occasions, namely, on the 63rd and 65th days, and were present in very small numbers. The goat was still alive on the 149th day, and appeared to be in normal health. No symptoms of disease were observed.

TABLE VI.—Goats inoculated with the *Nigerian strain.*

Animal	Inoculation	Day on which parasites were first seen in the blood	Day on which death occurred	Remarks
Goat, No. 11	Subcutaneous from guinea-pig, No. 1	63rd	—	Parasites only found twice, on the 63rd and 65th days, in very small numbers. Animal alive and well after 149 days.
Goat, No. 12	Subcutaneous from guinea-pig, No. 1	—	94th	Parasites never found in the blood. Animal died on the 94th day of a disease of the liver.

The above experiments indicate the slight degree of pathogenicity of the Nigerian trypanosome. Monkeys, guinea-pigs, and mice appeared to be the most susceptible animals, but even in them the disease was of an exceptionally chronic type.

Dogs were infected only with difficulty, and rats and goats seemed to be even more resistant. Of the 38 animals inoculated so far, only one has died of trypanosomiasis, and only ten have been infected. In some of the latter trypanosomes have not been found in the blood during the last two months.

TABLE VII.—Results obtained by various workers

	Yorke		Royal Society Commissioners in Uganda		Thomas and Linton	
	Incubation	Duration	Incubation	Duration	Incubation	Duration
Rat	Days 4	Days 14	Days 20	Months 3–8 +	Days 8	Days 45–388
Mouse	—	—	—	—	4–7	11–14
Guinea-pig	15	3–5 + months	—	—	12–45	3–8 months
Rabbit	12	3–5 + months	—	—	5–15	54–128 days
Dog	14	More than 1½ months	22	Some months	5–10	33–43 days
Monkey—						
Macacus rhesus	4	1–3	9–22	2–8	—	—
Cercopithecus callitrichus	13	3–5 months	9–40	2½–12 months	—	—
Cercopithecus ruber ...	—	—	—	—	—	—
Goat	Parasites only found once on 19th day in Goat 1; never found in Goat 2	3–5 + months	Parasites found on 13th day after 5th inoculation in one goat. Other two goats negative	One animal died on 22nd day. Other two recovered	—	All died; duration not stated
Donkey	—	—	Three were inoculated, but did not become infected	—	Parasites found on rare occasions	—
Horse	—	—	—	—	30 days	Recovered

G. – *T. gambiense*, from Gambia.
U. – Tryp. of Sleeping Sickness in Uganda.
K. – Tryp. of Sleeping Sickness in Congo.

with different strains of human Trypanosomes.

Laveran		Brumpt and Wurtz		Beck		Bentmann and Günther	
Incubation	Duration	Incubation	Duration	Incubation	Duration	Incubation	Duration
Days G. 13 U. 11 K. —	Days 62 77 81	Days 3–4	Months 4–5	Days 3–5	Months 3–4	Days U. 4·9 K. 12·5	Days 69 81
G. 13 U. 8–20 K. 30–45	57 153–216 281–351	2–3	3–4	2–3	8–10 days	U. 5·3 K. 4·9	49 90 —
G. 26 U. 16 K. 20	100 81 100	Always negative	1½	2–3	3–5 months	U. 6 experiments with no positive result. K. Only 2 positive results out of 21 experiments.	
—	—	Always negative	1–4 +	8–14	4–5 months	U. 9 days K. 9	28·6 days 49
—	—	17	66 days	2–3	1½–4 months	—	—
—	—	8–9	16 days to 2 months	—	—	—	—
—	—	7	19–88 + days	—	—	U. 10 K. 12	82 105
—	—	7	24	—	—	—	—

Very dissimilar results have been obtained by different authors working with various strains of *T. gambïense*. I have been unable, in Nigeria, to consult all the original papers on this subject; but the summary given by Yorke,[3] and reprinted above (Table 7), shows clearly how greatly the virulence may vary. The results of my experiments with the Nigerian strain are also shown in tabular form (Table 8) for comparison with those of other investigators.

TABLE VIII.—Results of inoculations with the *Nigerian strain*

Animal	Incubation	Duration	Remarks
Rat	59 days	69 + days	A single trypanosome seen once, on the 59th day, in one out of eight animals inoculated.
Mouse ...	5–9 days	107 + days	Three positive results out of seven experiments.
Guinea-pig	27 days	158 days	Three positive results out of twelve experiments.
Dog ...	85 days	115 + days	One positive result out of seven experiments.
Monkey ...	8–11 days	137 + days	
Goat ...	63 days	149 + days	

Several conditions affect the results of inoculations into animals. The virulence may be modified by a number of passages through the same species (Laveran,[4] Uhlenhuth,[5] &c.), inoculations may fail owing to the insufficient dilution of the infective blood (Uhlenhuth, Huebener and Woithe) or to too large a quantity being employed, and individual animals of the same species vary greatly in their susceptibility. If, as must be assumed, all the strains employed were of the same species, the different results obtained can only be explained by supposing that in some cases modification of the virulence had occurred in some such manner.

The strain of *T. gambiense* used by Yorke[6] was much more pathogenic than the Nigerian trypanosome to practically all the animals with which experiments were performed. His strain had been obtained from a case of human trypanosomiasis from the French Congo, in 1905, and had been maintained by passages through rats. It was said to have 'gradually become more virulent for rats.' The Nigerian trypanosome differs from all the other strains in its slight degree of pathogenicity to rats and dogs. Its virulence is unlike that of any of the strains of *T. gambiense* mentioned in the above summary, a fact which furnishes additional evidence in favour of considering it a new species of human trypanosome.

REFERENCES

(1) **Macfie.** Ann. Trop. Med. and Parasit., Vol. VII, No. 3a, August, 1913.

(2) **Beck.** Experimentelle Beiträge zur Infektion mit *Trypanosoma gambiense* und zur Heilung der menschlichen Trypanosomiasis. Arb. a. d. Kais. Gesund., Sec. 318, 1910.

(3) **Yorke.** Ann. Trop. Med. and Parasit., Vol. IV, No. 3, pp. 351-368, December, 1910.

(4) **Laveran** (A.). Influence des passages par cobayes sur la virulence de quelques trypanosomes. Bull. Soc. Path. Exot., 1908, 1, No. 4, p. 198.

(5) **Uhlenhuth, Hübener, und Woithe.** Experimentelle Untersuchungen über Dourine mit besonderer Berucksichtigung der Atoxylbehandlung. Arb. a. d. Kais. Gesund., 1908, XXVII, p. 256.

(6) **Yorke.** Loc. cit.

A NOTE ON THE OCCURRENCE OF SPIROCHAETOSIS OF FOWLS IN SOUTHERN NIGERIA

BY

J. W. SCOTT MACFIE, M.A. (Cantab.), M.B., Ch.B. (Edin.)
(WEST AFRICAN MEDICAL STAFF)

AND

J. E. L. JOHNSTON, M.B., B.S. (Lond.), D.T.M. & H. (Cantab.)
(WEST AFRICAN MEDICAL STAFF)

PLATES II, III

(*Received for publication* 12 *December*, 1913)

Spirochaetosis of fowls, first described by Marchoux and Salimbeni,[1] in Brazil, and subsequently studied by a number of workers, notably by Balfour,[2] occurs in many countries. In Africa the disease has been recorded in Khartoum by Balfour,[3] in Southern Rhodesia by Bevan,[4] in the neighbourhood of Tunis by Comte and Bouquet,[5] in Senegal and Somaliland by Brumpt,[6] in the French Soudan by Bouet,[7] in the vicinity of Cape Town by Jowett,[8] and in the German Cameroons by Mohn.[9] But so far as we are aware, its occurrence in British West Africa has not hitherto been chronicled.

In August, 1913, it was noticed that a fatal disease was attacking the poultry kept at Iju, near Lagos, in Southern Nigeria. Both ducks and fowls were dying off rapidly, and from the symptoms described, it was suspected that the disease would prove to the spirochaetosis. Thanks to the kindness of Mr. Harris, we were able to examine the bodies of several of the birds that had succumbed, and later on to study the disease in both fowls and Muscovy ducks.

On making enquiries in the neigbourhood, it was found that the disease was well known to the natives. Poultry rearing is almost universal in the villages of Nigeria, and all the natives questioned regarding the disease professed to be well acquainted with it. They recognised that when once it appeared it spread rapidly amongst their fowls, but they did not associate the disease with any particular condition, unless it were with the cold weather that accompanies the rainy season. Their treatment consisted in rubbing the legs of the infected birds with shea butter, a measure that had proved quite unsuccessful in the cases that came under our notice.

SYMPTOMS

The symptoms observed were those generally described in this disease, and were fever, diarrhoea, anaemia, somnolence, and paresis. The accompanying photographs (Plate II) illustrate the aspect of the infected birds At first they showed only slight symptoms of illness although the blood might be swarming with spirochaetes. The comb appeared to be unusually pale, there was slight diarrhoea, and the birds showed a tendency to stand for long periods with their feathers ruffled and their heads sunk between their shoulders. When they moved, their gait was staggering. Later the anaemia deepened, somnolence became a conspicuous feature, and paralysis of the wings, and especially of the legs, followed. Finally, the birds were unable to stand, and lay prone on the ground until death occurred. The appetite remained good almost up to the end.

The disease first showed itself in some Muscovy ducks, but subsequently spread to the fowls kept in the same compound. Some of the birds attacked were chickens. All the infected fowls were thoroughly searched for ticks and other parasites, but none were found with the exception of a few fleas on the combs which have been identified by Mr. N. C. Rothschild as ***Echidnophaga gallinaceus***.

POST-MORTEM APPEARANCES

After death the bodies of the birds were found to be wasted, and the tissues unnaturally pale. The spleen was always considerably enlarged. The most conspicuous feature was, however, the condition of the liver which was greatly enlarged, and in which necrotic patches occurred sometimes of considerable size. In one case a large blood clot was found in the stomach.

MORPHOLOGY OF THE PARASITE

But little need be said about the morphology of the spirochaete, since it appeared to be very similar to that described by Balfour in the case of the Sudanese strain. The general appearance of the parasites is shown on Plate III (Figs. 1, 2). The spirochaetes varied greatly in length, and in the number of their spirals. Some forms were as short as 9μ, and had only three or four spirals, others were as long as 27μ or more, and had twelve or fourteen spirals. The latter were of the combined type, and appeared to consist of two spirochaetes connected by a very fine filament. In some cases the spirals were close, in others they were open. One end of the body was often curled up. The bodies of the spirochaetes were pointed at each end, and in some individuals chromatic dots were clearly visible. In others breaks or gaps occurred in the central core. The breadth of the body varied considerably, some of the parasites being very much stouter than others. Some forms which appeared at first sight to be stout individuals proved on more careful examination to be made up of two spirochaetes. The appearance suggested the result of a recent longitudinal division.

BLOOD CHANGES

In addition to the condition of the blood described above, certain changes in the leucocytes were observed. Large vacuolated mononuclear cells were commonly met with, and ingestion of red blood corpuscles was seen to have occurred. Both these phenomena have been previously observed by Levaditi,[10] and need not be further discussed. Figs. 3 to 6 on Plate III illustrate the condition described.

INOCULATION INTO MAMMALS

Marchoux and Salimbeni found that guinea-pigs were insusceptible to the parasite of Brazilian septicaemia of fowls, and Balfour failed to infect gerbils by inoculations with the Sudan strain. The latter author concludes that 'this is an avian spirochaetosis, non-transmissible to mammals.' But Levaditi and Lange have shown that fowl spirochaetes may produce a benign infection in rabbits, and Deutz[11] has succeeded in infecting mice.

In our experiments one rat was inoculated subcutaneously with blood from a Muscovy duck, in which countless spirochaetes were present. The animal never showed the parasites in its blood, and no signs of spirochaetal infection were observed.

ACTION OF ATOXYL AND SALVARSAN

Both atoxyl and salvarsan have been proved to give satisfactory results in the treatment of spirochaetosis of fowls in other countries. In our investigations we employed the former drug in three cases, and the latter in two.

Atoxyl. Three fowls were treated by subcutaneous injections of 3/50 gr. atoxyl.

Fowl 99. On August 29th, 1913, the first injection was given. At this time the fowl showed definite symptoms of the disease. The comb was very pale, there was diarrhoea and somnolence, and the gait was unsteady. Spirochaetes were numerous in the blood, and some clumps were present. The next day no parasites could be found in the blood, and they did not reappear subsequently. The inoculation was repeated on the 4th and 7th days. By the 11th day the bird seemed to have regained its normal condition, and a month later was still apparently quite healthy.

Fowl 100. A young bird showing well-marked symptoms—anaemia, ruffled feathers, diarrhoea and uncertain gait. Spirochaetes scanty. The first injection was given on August 31st, and was repeated on the 3rd and 6th days. No spirochaetes were seen after the first injection, and the bird rapidly recovered, and appeared to be in normal health by the 10th day.

Fowl 101. A young bird showing well-marked symptoms. Spirochaetes very numerous in the blood. The first injection was given on August 31st, and the dose was repeated on the 3rd and 6th days. Spirochaetes continued to be present in the blood until the 6th day. The day after the second injection the blood was found to contain masses of tangled spirochaetes. No parasites were seen on subsequent days, but the bird continued to show symptoms of the disease, and died on the 11th day after the first injection.

In the first two cases atoxyl appeared to effect the cure of the disease. In the third, either the condition had advanced too far before treatment was begun, or the dose of atoxyl was insufficient to destroy the parasites before they had effected mortal injuries on the host. In spite of the injections the disease progressed to the so-called 'crisis,' the spirochaetes agglutinated into clumps, and vanished from the peripheral blood, and eight days later the bird died.

Salvarsan. Two fowls were treated with subcutaneous injections of salvarsan triturated with oil. The dose employed was in each case 0·002 gm.

Fowl 97. The fowl showed well-marked symptoms of spirochaetosis, and the blood was swarming with parasites. The first injection was given on August 30th, and was repeated on the 4th and 7th days. A few spirochaetes were found in the blood on the day after the first injection, but none was detected subsequently. During the first few days the paralysis of the legs and the wings progressed until the bird was unable to stand, then some improvement began to take place so that the fowl was able to move about unsteadily by the 11th day. The other symptoms began to improve simultaneously. Six weeks later the bird appeared to be in good health in every respect excepting that the paresis of the wings and legs persisted. No marked improvement in this latter symptom has taken place during the last month.

Fowl 98. The bird appeared to be in an advanced stage of the disease, it was quite unable to stand, and lay helpless on the ground. The blood contained countless spirochaetes. The first injection of salvarsan was given on August 30th, and the dose was repeated on the 4th and 7th days. Spirochaetes were still numerous the day after the first injection, and a few were present on the third day. From this time onwards no more parasites were seen. The condition of the bird began to show definite improvement from the 6th day, and by the 11th day recovery was well nigh complete. Six weeks later the fowl appeared to be in perfect health.

In both these cases salvarsan appeared to save the life of the bird. In the case of Fowl 98, this result was remarkable considering the stage to which the disease had advanced before treatment was begun, and the failure of atoxyl to prevent death in the case of Fowl 101, which was not in so serious a condition. This observation corresponds with those of Dschunkowsky,[12] who found salvarsan to be more active than atoxyl in the treatment of spirochaetosis of geese. The persistence of paresis after recovery from spirochaetosis was observed as a sequel to the illness by Marchoux and Salimbeni.

THE 'AFTER PHASE' BODIES OF BALFOUR

Balfour has described certain appearances in the red blood corpuscles of fowls suffering from spirochaetosis which he has termed 'after phase bodies,' and which he considers represent a stage in the development of the parasite. They occur especially during the post-critical stage of the disease, at a time when free spirochaetes cannot be detected in the blood by the ordinary methods of examination. They have been described by Jowett in the spirochaetosis in the vicinity of Cape Town, as well as by Balfour in the Sudanese strain.

We have naturally looked for these bodies with considerable care in the cases that we have studied in Nigeria. We have not, however, succeeded in convincing ourselves of their presence. In a few corpuscles we have seen bodies not unlike those figured by Balfour, but they have shown little or no structure, and might be accounted for by some other cause. Even if these bodies were the 'after phase bodies' of Balfour, they occurred in such small numbers as to constitute a marked difference between the Sudanese and Nigeria strains.

Several species of parasites seem to be concerned in the condition known generally as spirochaetosis of fowls. The parasite of the Brazilian septicaemia, named by Stephens and Christophers *Sp. gallinarum* (= *Sp. marchouxi*, Nuttall),[13] appears to be the most common, and is by some authors considered to be the only species. Brumpt, however, has distinguished the Tunisian strain and the Senegal strain from this species on account of immunity reactions, and has named the former *Sp. nicollei*, and the latter *Sp. neveuxi*. Balfour also has reluctantly decided that the Sudan strain constitutes a new species for which he proposes the name *Sp. granulosa*. In addition to these species, the spirochaete found by Sakharoff attacking geese in the Caucasus has been called *Sp. anserina*. This parasite is, however, capable of infecting fowls, producing in them a disease similar to that caused by *Sp. marchouxi*.

We have been unable to investigate the immunity reactions of the spirochaete infecting poultry in Nigeria, so that it is impossible to determine exactly the species to which it belongs. Morphologically, the parasite appears to be identical with *Sp. marchouxi*, and the symptoms of the disease, and the post-mortem appearances are similar to those described in the case of Brazilian septicaemia of fowls. In several respects the disease differs from that described by Balfour in Sudanese fowls.

It would not be unnatural to expect that fowl spirochaetosis in Brazil and in West Africa might be due to the same species of parasite. In the days when slaves were exported from West Africa there must have been constant communication between the two countries, and it is not unlikely that fowls, some of which may have been infected with spirochaetes, were carried over to Brazil. It is interesting to note in this connection that Blaizot,[14] as quoted by Balfour, believes that from a study of the question of immunity, 'it may yet be possible to affirm that Africa is the original country whence avian spirochaetosis spread.'

REFERENCES

1. Marchoux, E., and Salimbeni, A. (1903). La Spirillose des Poules. Ann. de l'Inst. Pasteur, Vol. XVIII, pp. 569-580.

2. Balfour, A. Spirochaetosis of Sudanese Fowls. Third Report of the Wellcome Research Laboratories, 1908, and Fourth Report of the Wellcome Research Laboratories, 1911.

3. Loc. cit.

4. Bevan, Ll. E. W. (1908). Spirillosis of Fowls in Southern Rhodesia. Journ. of Comp. Path. and Therap., Vol. XXI, Part 1.

5. Comte, C., and Bouquet, H. (1909). Recherches experimentales sur la spirillose des poules en Tunisie. Arch. de l'Inst. Past. de Tunis, p. 163.

6. Brumpt, E. (1909). Sur une nouvelle spirochétose des poules du Sénégal produite par *Spirochaeta neveuxi*, n.sp. Bull. de la Soc. de Path. Exot., Vol. II, p. 285. And Existence d'une Spirochétose des Poules ***Spirochaeta gallinarum*** dans les pays Somali. Compt. Rend. Soc. Biol., Vol. LXVII, p. 174.

7. Bouet, G. (1909). Spirillose des Poules au Soudan Français. Bull. Soc. Path. Exot., Vol. II, p. 288.

8. Jowett, W. (1910). Fowl Spirochaetosis, Note on the Occurrence of, at the Cape. Agricult. Journ. of the Cape of Good Hope, Vol. XXXVII, No. 6.

9. Mohn (1909). Über Hühnerspirochätose in Kamerun.

10. Levaditi, G. (1904). Contributions à l'Étude de la Spirillose des Poules. Ann. de l'Inst. Past., Vol. XVIII.

11. Deutz (1912). Ueber Versuche zur Uebertragung von Huehnerspirochaeten auf Mause. Hyg. Rundschau, Vol. XXII, No. 16. Quoted in Trop. Vet. Bull., Vol. I, No. 2, p. 104.

12. Dschunkowsky, E. (1911). Heilversuche mit Ehrlich-Hata '606' bei der Gänsespirillose, etc. Berl. tierarzth. Woch.

13. Fantham, H. B. (1911). Some researches on the life-cycle of Spirochaetes. Annals Trop. Med. & Parasitol., V, p. 479.

14. Blaizot, L. (1910). Note sur la récurrence dans la Spirochétose des Poules en Tunisie (virus de Degache). Arch. de l'Inst. Past. de Tunis, Part 2, p. 53. Quoted by Balfour in the Fourth Report of the Wellcome Research Laboratories.

EXPLANATION OF PLATES

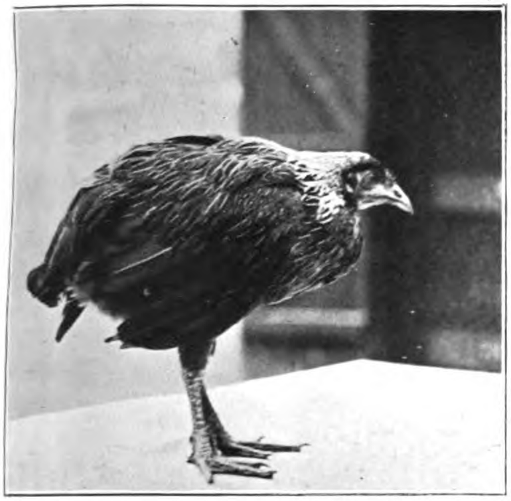
Avian spirochaetosis.

PLATE II

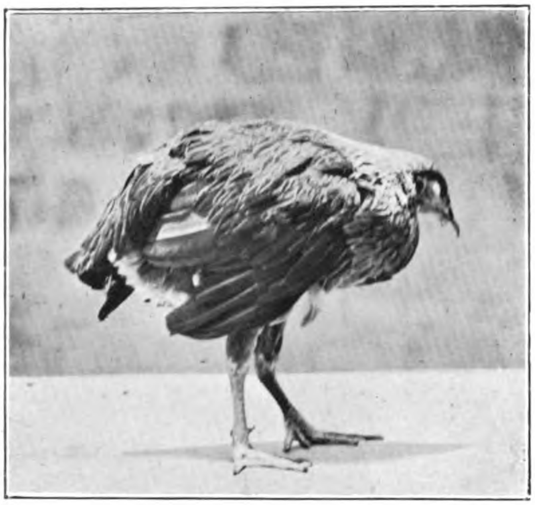
Fig. 1, 2, 4, and 5. Fowls in various stages of the disease. Note the attitude, the ruffled feathers, the somnolence, and the paresis of the wings and legs.

Fig. 3. Muscovy duck in a late stage of the disease, and unable to stand on its legs.

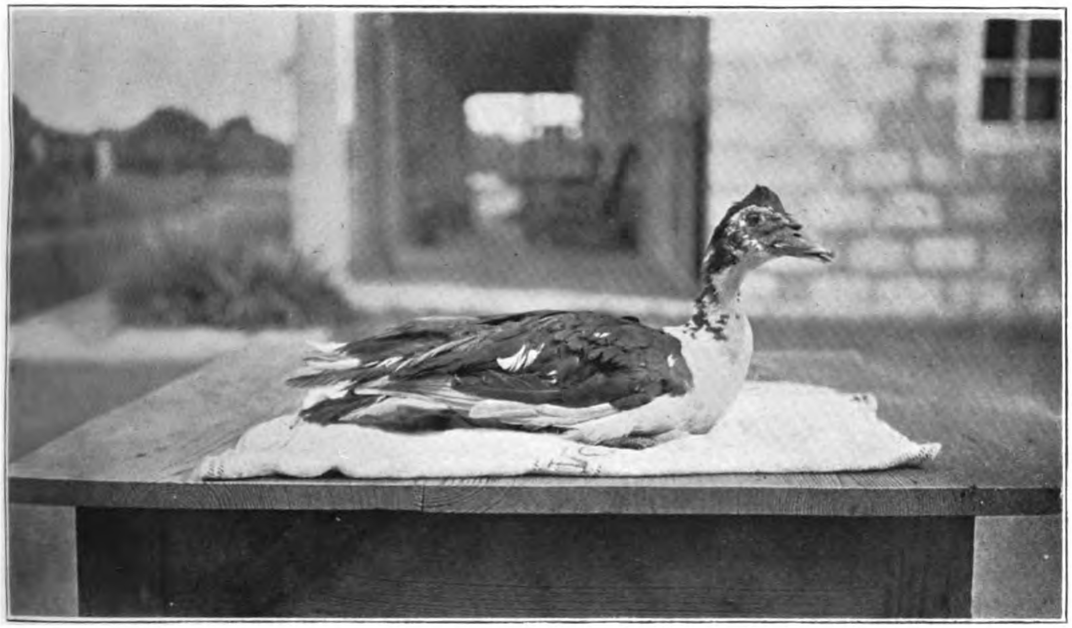
PLATE II



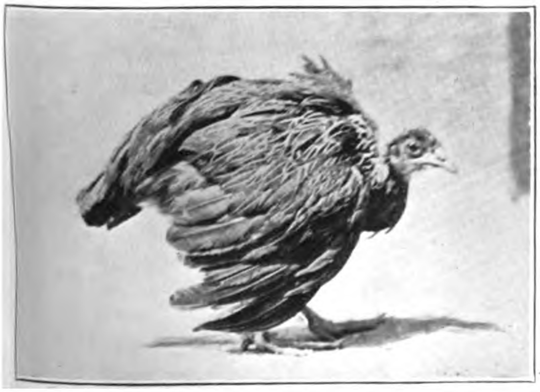
1



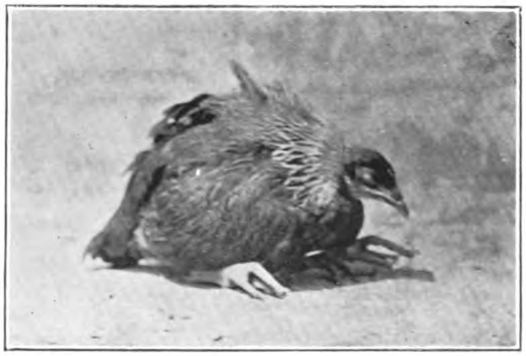
2



3



4



5

C. Tinling & Co., Ltd., Imp.

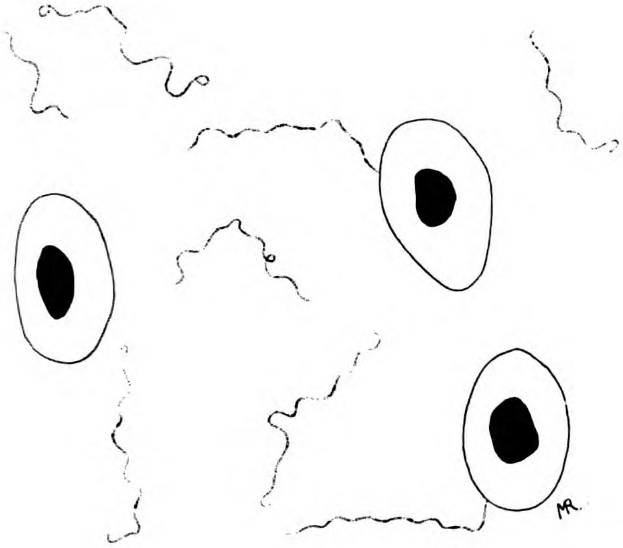
PLATE III

Fig. 1. Spirochaetes, long and short, some with few, some with numerous spirals. Chromatin dots seen in some parasites.

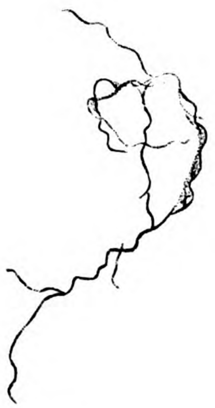
Fig. 2. Group of coiled Spirochaetes.

Figs. 3, 4. Vacuolation of mononuclear leucocytes.

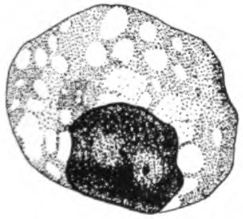
Figs. 5, 6. Auto-erythro-phagocytosis in duck's blood.



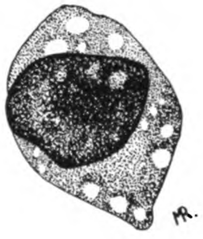
1



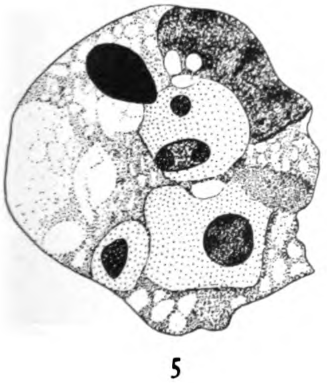
2



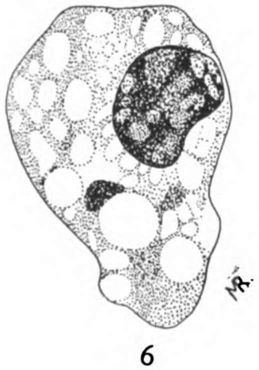
3



4



5



6

C. Tinling & Co., Ltd., Imp.

OBSERVATIONS ON A SO-CALLED CURE FOR TRYPANOSOMIASIS

BY

WARRINGTON YORKE

AND

B. BLACKLOCK

(From the Runcorn Research Laboratories of the Liverpool School of Tropical Medicine)

(Received for publication, 13 *January,* 1914)

The experiments of which an account is given in this paper were undertaken at the request of the British South Africa Company, who had been approached by Mr. H. C. Sieg, with a view to the purchase by them of a serum reputed to be of great value in the treatment of trypanosomiasis.

It would appear from correspondence between His Excellency the Governor-General and the Prime Minister of the Union of South Africa that in May and June, 1912, certain experiments were carried out by Professor Jolly, at the South African College, Capetown. The object of these experiments, which were not completed, was to test the claims of Dr. Menharto, with whom Mr. Sieg was associated, with regard to the therapeutic properties of a serum supposed to cure Sleeping Sickness.

In a letter of the Prime Minister of the Union of South Africa, dated March 26, 1913, it is stated that Mr. Sieg and Dr. Menharto 'were not prepared to divulge the nature of the serum employed by them or the process by which it was obtained, and as the experiments they desired the Government to undertake on their behalf were estimated to cost a sum of possibly £5,000, ministers were not able to consider the matter.'

In view of the publicity which had been accorded this serum as a cure for Sleeping Sickness and of the apparently promising results obtained by Professor Jolly in his incomplete experiments with Dr. Menharto's serum, we decided, in spite of the fact that Mr. Sieg would not inform us either of the constitution or the method of preparation of the 'serum,' to accede to the request of the British South Africa Company, and examine the remedial powers of this serum on animals experimentally infected with trypanosomiasis.

EXPERIMENTS

We were supplied with several different 'sera' by Mr. Sieg for trial. The 'sera' were grouped in various grades. It was stated by Sieg, in a letter to us, 'that sera were prepared by a mixture of

TABLE I.—Giving the results of treating small animals infected with various pathogenic trypanosomes with the first supplies of 'serum' obtained from Mr. Sieg.

No.	Animal	Strain	No. of Injections of serum given	Day of death	Remarks
1	Guinea-pig	*T. rhodesiense* ...	2	41	Course of disease unaltered.
2	,,	,,	3	17	,, ,,
3	,,	,,	4	35	,, ,,
4	Rabbit	,,	2	18	,, ,,
5	,,	,,	2	24	,, ,,
6	,,	,,	2	16	,, ,,
7	Rat	,,	2	11	,, ,,
8	,,	,,	2	8	,, ,,
9	,,	,,	2	8	,, ,,
10	Guinea-pig	*T. ugandae*	2	24	,, ,,
11	,,	,,	7	32	,, ,,
12	,,	*T. evansi*	7	58	,, ,,
13	,,	,,	6	22	,, ,,
14	,,	,,	7	46	,, ,,
15	,,	,,	9	55	,, ,,
16	,,	*T. gambiense* ...	7	68	,, ,,
17	,,	,,	7	59	,, ,,
18	Rat	*T. brucei*	1	5	,, ,,
19	,,	,,	1	5	,, ,,
20	,,	,,	1	5	,, ,,

Table II.—Giving the results of treating small animals infected with various pathogenic trypanosomes with the second supplies of 'serum' obtained from Mr. Sieg.

No.	Animal	Strain	No. of injections of serum given	Day of death	Remarks
1	Rabbit	*T. brucei*	1	11	Course of disease unaltered.
2	,,	,,	1	24	,, ,,
3	,,	,,	1	23	,, ,,
4	,,	,,	1	12	,, ,,
5	,,	,,	1	11	,, ,,
6	Guinea-pig	,,	1	17	,, ,,
7	,,	,,	1	9	,, ,,
8	,,	,,	1	18	,, ,,
9	,,	,,	1	17	,, ,,

certain blood plasma, and contained no chemical or mineral poisons in them.' Injection was to be made subcutaneously, and the dose recommended was 1 c.c. for small animals, to be repeated if necessary.

We employed the sera as directed in the treatment of 29 animals—rats, guinea-pigs and rabbits—infected with various strains of pathogenic trypanosomes, including *T. gambiense* and *T. rhodesiense*. Details are given in the tables. In no case did recovery take place nor, in fact, was the course of the disease influenced in the slightest degree.

CONCLUSION

The 'sera' supplied to us by Mr. H. C. Sieg had not the slightest therapeutic value in animals experimentally infected with pathogenic trypanosomes.

ANTIMONY TRIOXIDE IN THE TREATMENT OF EXPERIMENTAL TRYPANOSOMIASIS

BY

WARRINGTON YORKE

AND

B. BLACKLOCK

(From the Runcorn Research Laboratories of the Liverpool School of Tropical Medicine)

(Received for publication 13 *January,* 1914)

In May, 1913, Kolle, Hartoch, Rothermundt and Schürmann published a preliminary report* of their investigations on the value of various antimony compounds in the treatment of experimental trypanosomiasis. A further account† of this work was published in August, 1913. As a result of an exhaustive examination of many antimony compounds—inorganic and organic—the authors arrived at the conclusion that in the trioxide they had discovered a remedy which, in respect of its chemo-therapeutic index and its permanent sterilising effects, far surpassed all others hitherto employed in the therapy of trypanosomiasis. It was with the object of examining this statement, and ascertaining if it applied in the case of larger animals, that the experiments recorded in this paper were performed.

In the earlier experiments the smaller laboratory animals (rabbits, guinea-pigs and rats) infected with various strains of trypanosomes were treated with one or more injections of antimony trioxide.

---

*Ueber neue Prinzipien und neue Präparate für die Therapie der Trypanosomeninfektionen. Deut. Med. Wochenschr., 1913, May 1, Vol. XXXIX, No. 18, p. 825.

†Chemotherapeutische Experimentalstudien bei Trypanosomeninfektionen, Zeitschr. für Immunitatsf. und experiment. Therapie, 1913, August 5, Vol. XIX, No. 1, p. 66.

METHODS OF ADMINISTRATION

The drug was administered intramuscularly as a rule, but intraperitoneal and intravenous injection were also used in some cases. For the intramuscular injections 10 per cent. and 40 per cent. suspensions* of the drug in oil were prepared; for the intraperitoneal injections 1 per cent. suspension in 0·9 per cent. sodium chloride solution was used. As the latter suspension quickly deposited, it was found unsuitable for intravenous injection. In order to overcome as far as possible this difficulty, the drug was reduced by special means to an exceedingly fine powder which was suspended in distilled water. The suspension was allowed to stand in a cylinder for 18 hours, after which time the upper portion was decanted off. The amount of antimony trioxide contained in these suspensions was found to vary from 0·13 to 0·35 per cent. We are indebted to Mr. Prosper H. Marsden for kindly making this preparation.

The intramuscular method of administration possessed distinct advantages over the others, and was followed by much more beneficial results. By whatever method the drug was given, the amount absorbed was exceedingly small. Following intramuscular injection, the drug was found apparently unaltered, at the site of inoculation, six months later. In those animals in which it was given intraperitoneally yellowish white nodules consisting of small encapsuled masses of the drug were found in large numbers situated in the mesentery and omentum. Intravenous injection resulted in a deposit of the drug in the vein of the ear of rabbits used for injection, with the result that the vessel became plugged. This was due to the fact that the suspension in distilled water immediately deposited on contact with a fluid containing salt in solution.

One great disadvantage of the intramuscular method of injection was the frequency with which sterile abscesses formed. These, although rare in small animals, were very frequent in dogs and donkeys. In the latter animals even exceedingly small doses gave rise to abscesses; goats did not develop abscesses except after very large injections. Intravenous injection of the drug into the ear veins of rabbits gave rise to plugging as mentioned above, and dry gangrene sometimes resulted with sloughing of portions of the ears. No ill effects were observed after intraperitoneal administration.

---

* We are indebted to Professor Kolle for kindly furnishing us with a supply of 'Trixidin.' This was used by us in our earlier experiments; later we prepared our own suspensions.

TABLE I.—Giving results of injecting normal animals with a suspension of Antimony trioxide.

No.	Animal	Amount of Sb_2O_3 given, in mg. per 10 g. of body weight	Day of death	Remarks
37	Donkey	0·2	15th	The drug was given in one place.
56	Dog	1·0	3rd	The drug was given in four different places.
57	,, ...	0·5	5th	The drug was given in two different places.
97	,, ...	1·0	8th	The drug was given in four different places.
98	,, ...	1·0	17th	The drug was given in one place.
99	,, ...	1·0	2nd	The drug was given in two places.

TOXIC EFFECTS OF THE DRUG

Small animals appear to be much more tolerant of the drug than large ones. In guinea-pigs, rabbits, rats and mice 1 mg. per 10 grms. of body weight could be given intramuscularly or intraperitoneally at a single injection without any ill effect, either local or general. Frequently very much larger doses were administered without any appreciable toxic effect. For example, in Table VIII it will be seen that Guinea-pig 8 received at a single injection 20 mg. per 10 grms. body weight, and remained alive for 40 days. The weight of the animal was unchanged until shortly before death. There was no abscess formation, and large quantities of the drug were found at the time of death, distributed between the muscle fibres at the site of inoculation. The drug was given prophylactically, and the animal inoculated subsequently with *T. rhodesiense*. We were unable to determine the cause of death, as trypanosomes were never found in the blood, and an animal inoculated from the guinea-pig at the time of death did not become infected. Many other examples of animals which lived for considerable periods after having received a single large dose of the drug are shown in the tables. We cannot state what is the largest amount that can be given to small laboratory animals without causing toxic effects, but it is certainly much more than 1 mg. per 10 grms. body weight. It must be remembered, however, that only a minute fraction of the amount injected is absorbed, the vast proportion remaining unaltered at the site of inoculation. This probably accounts for the slight toxicity. Possibly the irregularity of the rate of absorption from the local depôt may explain the contradictory results obtained, both as regards host and parasites. On the other hand large animals, e.g., donkeys and dogs, are much more susceptible to the drug. Death resulted in several cases after the administration of doses of 1 mg. or less per 10 grms. body weight. Goats appear to tolerate the drug better.

TABLE II.—Giving the results of treatment with Antimony trioxide of small animals infected with *T. rhodesiense*.

No.	Animal	Day of treatment	Amount of Sb_2O_3 given, in mg. per 10 g. of body weight	Day on which trypanosomes disappeared from the blood	Day of relapse	Day of death	Remarks
7	Guinea-pig ...	15th	5·0	16th	...	31st	No relapse; animal died negative.
16	,,	8th	1·0	10th	...	111th	,, ,,
39	,,	10th	1·0*	11th	32nd	...	
		32nd	1·0*	33rd	...	84th	No second relapse; animal died negative.
40	,,	10th	1·0	12th	...	65th	No relapse; rat inoculated at time of death did not become infected.
41	,,	10th	0·8	11th	...	67th	No relapse; animal died negative.
42	,,	10th	1·0	12th	...	86th	,, ,,
76	,,	18th	1·0	20th	...	134th	,, ,,
77	,,	18th	1·0	22nd	...	85th	No relapse; rat inoculated at time of death did not become infected.
79	,,	13th	1·0	15th	117th	...	
		117th	2·0	118th	...	...	Animal alive and well on 137th day.
78	,,	13th	1·0	15th	...	120th	Trypanosomes never found in blood, but rat inoculated on 113th day became infected.
81	Rabbit	7th	1·0†	8th	27th	54th	

*Intraperitoneal injection of 1 per cent. suspension in saline solution.

†Injected intramuscularly in two separate regions.

THERAPEUTIC ACTION OF THE DRUG

The value of the drug was examined for a large number of different strains of trypanosomes, and remarkable variations in susceptibility to its effects were exhibited. As will be seen from Table II, a single intramuscular injection of 1 mg. per 10 grms. of body weight invariably sufficed to clear the trypanosomes from the peripheral blood, of guinea-pigs and rabbits infected with *T. rhodesiense*. As a rule there was no relapse, the animals living for periods varying from 34 to 134 days.

TABLE III.—Giving results of treatment of small animals, infected with *T. gambiense*, with Antimony trioxide.

No.	Animal	Day of treatment	Amount of Sb_2O_3 given, in mg. per 10 g. of body weight	Day on which trypanosomes disappeared from the blood	Day of relapse	Day of death	Remarks
4	Guinea-pig ...	44th	1·5	...	...	...	Trypanosomes almost invariably present in peripheral blood in large numbers.
		61st	0·6	...	...	...	
		67th	2·0	...	...	...	
		80th	0·6*	...	...	86th	
5	,,	26th	1·4	31st	...	38th	No relapse; animal (rat) inoculated at time of death did not become infected.
14	Rat	5th	4·0	...	...	14th	Trypanosomes always numerous in blood.
33	Guinea-pig ...	26th	2·0	...	...	...	
		32nd	1·6	35th	...	57th	No relapse after second inoculation; rat inoculated at time of death did not become infected.
53	Rat	6th	1·0*	...	...	...	
		11th	1·0*	...	...	...	
		15th	2·0	...	...	...	
		18th	1·0*	...	...	...	
		28th	2·0	...	...	...	
		37th	3·0	...	...	41st	Trypanosomes always present in peripheral blood in large numbers.
54	,,	6th	1·0*	...	...	...	
		11th	1·0*	...	...	...	
		15th	2·0	...	...	...	
		18th	1·0*	...	...	...	
		28th	2·0	...	...	30th	,, ,,
74	,,	3rd	2·0	...	...	...	
		5th	2·0	...	...	...	
		9th	4·0	...	...	16th	,, ,,

*Intraperitoneal injection of 1 per cent. suspension in saline solution.

Very different results were obtained on treating guinea-pigs and rats infected with *T. gambiense*. This strain[†] appears to be remarkably resistant to the drug, and in spite of the administration of relatively large and frequently repeated doses, the disease ran a normal course, trypanosomes generally being present in the peripheral blood in large numbers.

TABLE IV

A.—Giving the results of treatment of small animals, infected with *T. ugandae*, with Antimony trioxide.

No.	Animal	Day of treatment	Amount of Sb_2O_3 given, in mg. per 10 g. of body weight	Day on which trypanosomes disappeared from the blood	Day of relapse	Day of death	Remarks
6	Guinea-pig ...	25th	2	27th	164th	182nd	
18	Rat	7th	8	8th	...	15th	No relapse; animal died negative.

B.—Giving the results of treatment of small animals, infected with *T. brucei*, with Antimony trioxide.

No.	Animal	Day of treatment	Amount of Sb_2O_3 given, in mg. per 10 g. of body weight	Day on which trypanosomes disappeared from the blood	Day of relapse	Day of death	Remarks
13	Rat	3rd	6·5	5th	...	87th	No relapse; rat inoculated at time of death did not become infected.
34	Guinea-pig ...	22nd	1·0	...	...	26th	Trypanosomes did not disappear from blood.
44	Mouse	5th	1·0*	...	...	...	
		7th	2·5*	8th	...	24th	No relapse after second treatment; died negative.
45	..	5th	1·0*	...	...	...	
		7th	2·5*	8th	32nd	...	
		35th	5·0	37th	49th	...	
		49th	10·0	50th	79th	79th	

[†] This strain was received by us from Professor Mesnil of the Pasteur Institute. He informs us that its origin was a case of sleeping sickness contracted in the French Congo. It was obtained from the patient (G. Y.) on December 23, 1904, and has since been preserved in laboratory animals. (See Bull. Soc. Path. Exot., June, 1912.)

C.—Giving the results of treatment of rats, infected with *T. evansi*, with Antimony trioxide.

No.	Animal	Day of treatment	Amount of Sb_2O_3 given, in mg. per 10 g. of body weight	Day on which trypanosomes disappeared from the blood	Day of relapse	Day of death	Remarks
43	Rat	10th	1*	11th	36th	38th	
52	,, ...	17th	1*	18th	25th	...	
		27th	2	28th	...	48th	No relapse after second treatment; died negative.

D.—Giving the results of treatment of rats, infected with *T. equiperdum*, with Antimony trioxide.

No.	Animal	Day of treatment	Amount of Sb_2O_3 given, in mg. per 10 g. of body weight	Day on which trypanosomes disappeared from the blood	Day of relapse	Day of death	Remarks
19	Rat	11th	4	12th	...	...	Animal alive and well on 238th day; rat sub-inoculated on 214th day not become infected.
49	,, ...	18th	1*	20th	97th	122nd	

E.—Giving the results of treatment of small animals, infected with *T. congolense*, with Antimony trioxide.

No.	Animal	Day of treatment	Amount of Sb_2O_3 given, in mg. per 10 g. of body weight	Day on which trypanosomes disappeared from the blood	Day of relapse	Day of death	Remarks
11	Rabbit	21st	5·0	23rd	...	...	Animal alive and well on 255th day; rat sub-inoculated on 231st day did not become infected.
48	Rat	20th	1·0*	21st	35th	...	
		35th	2·0	36th	54th	...	
		70th	2·5	72nd	151st	153rd	
72	Rabbit	7th	0·3	...	...	...	
		11th	0·3	...	...	13th	Trypanosomes did not disappear from the blood.

F.—Giving the results of treatment of small animals, infected with *T. equinum*, with Antimony trioxide.

No.	Animal	Day of treatment	Amount of Sb_2O_3 given, in mg. per 10 g. of body weight	Day on which trypanosomes disappeared from the blood	Day of relapse	Day of death	Remarks
47	Guinea-pig ...	35th	1·0[*]	36th	...	115th	No relapse; animal died negative.
69	Rabbit	30th	1·0	31st	46th	...	
		46th	0·25	47th	50th	...	
		50th	1·0	...	...	52nd	

The results of treating small animals infected with various trypanosomes pathogenic to domestic stock, viz., *T. ugandae*, *T. brucei*, *T. evansi*, *T. equiperdum*, *T. congolense*, and *T. equinum* are grouped together in Table IV. While the course of the disease appears to have been influenced in almost all cases, only two of the animals have survived up to the time of writing. Some remained negative for long periods after treatment, and died without parasites reappearing in their blood, whilst others relapsed after being negative for as long as 134 days.

---

[*] Intraperitoneal injection of 1 per cent. suspension in saline solution.

Table V.—Giving the results of treating with Antimony trioxide, goats infected with *T. vivax*.

No.	Day of treatment	Amount of Sb_2O_3 given, in mg. per 10 g. of body weight	Day on which trypanosomes disappeared from the blood	Day of relapse	Day of death	Remarks
1	38th	1·0	...	...	...	
	44th	2·5	...	...	45th	Trypanosomes did not disappear from blood.
2	8th	1·0	10th	...	22nd	No relapse ; animal died negative.
12	7th	4·0	8th	...	13th	,, ,,
17	11th	1·0	13th	15th	24th	
29	15th	0·25	...	...	...	
	18th	0·25	...	...	...	
	21st	0·25	23rd	27th	...	
	26th	0·25	28th	...	...	
	30th	0·25	...	32nd	...	
	32nd	0·06	...	...	...	
	33rd	0·06	33rd	...	...	
	35th	0·06	...	...	...	
	38th	0·06	...	...	193rd	No relapse after 33rd day; animal died negative; diarrhoea.
31	16th	0·03	...	...	...	
	18th	0·03	19th	21st	...	
	21st	0·03	22nd	...	...	
	26th	0·03	...	...	...	
	30th	0·03	...	...	198th	No relapse after 22nd day; animal died negative.
60	6th	0·06	...	...	...	
	7th	0·06	...	...	...	
	8th	0·06	...	...	...	
	9th	0·06	11th	13th	...	
	14th	0·06	...	...	...	
	15th	0·06	...	...	...	
	16th	0·06	...	...	...	
	18th	0·06	...	...	...	
	20th	0·06	...	...	...	
	22nd	0·06	23rd	...	...	
	24th	0·06	...	...	...	
	26th	0·06	...	...	...	
	30th	0·06	...	...	...	
	35th	0·06	...	...	...	
	42nd	0·06	...	124th	...	
	125th	0·06	...	...	...	
	127th	0·06	127th	...	140th	
70	29th	0·06	...	...	...	
	30th	0·06	...	...	...	
	31st	0·06	...	...	...	
	34th	0·06	...	...	...	
	35th	0·06	35th	...	...	
	39th	0·06	...	...	...	
	40th	0·06	...	...	...	
	41st	0·06	...	...	...	
	47th	0·06	...	...	116th	No relapse ; animal died negative.
73	33rd	0·06	...	...	...	
	34th	0·06	...	...	...	
	35th	0·06	...	...	...	
	36th	0·06	36th	...	...	
	38th	0·06	...	...	...	
	41st	0·06	...	...	...	
	48th	0·06	...	...	...	
	51st	0·06	...	...	134th	No relapse ; animal died negative.

The results of treating goats infected with *T. vivax* are given in Tables V and X. The best effects were obtained by repeated administration of small doses of the drug. Most of those animals which received one or two relatively large doses died in a short time either with or without trypanosomes in the blood. In all of the seven goats treated with small and frequently repeated doses, the course of the disease was greatly modified, and life was considerably prolonged. As a rule trypanosomes did not definitely disappear till six or eight doses of the drug had been given. Five of these goats died negative after periods varying from 109 to 198 days. Of the other two, one relapsed on the 124th day, and in spite of two further injections of the drug, died with trypanosomes in its blood on the 140th day. The other relapsed on the 184th day; it was again treated by repeated small doses. Trypanosomes disappeared from the blood, and were not found again; the animal died negative on the 201st day. The strain of *T. vivax* employed kills goats on an average in 30 days.

TABLE VI.—Giving the results of treatment of donkeys, infected with various trypanosomes, with Antimony trioxide.

No.	Strain of trypanosomes	Day of treatment	Amount of Sb_2O_3 given, in grammes	Day on which trypanosomes disappeared from the blood	Day of relapse	Day of death	Remarks
30	*T. rhodesiense*	18th	1·6	...	...	...	
		21st	1·2	...	...	...	
		23rd	2·0	...	...	...	
		28th	2·0	...	...	...	
		31st	0·2	...	...	...	
		32nd	0·2	33rd	...	...	
		34th	0·2	...	...	...	
		36th	0·2	...	...	...	
		38th	0·2	...	...	...	
		41st	0·2	...	...	56th	No relapse; animal died negative.
32	*T. equiperdum*	22nd	1·0	...	...	...	
		25th	2·0	...	...	...	
		33rd	0·2	...	...	...	
		34th	0·2	35th	...	...	
		36th	0·2	...	...	...	
		37th	0·2	...	...	...	
		40th	0·2	...	...	...	
		43rd	0·2	...	...	...	
		94th	0·1	...	...	147th	On 141st day a rat was inoculated and became infected.
68	*T. equiperdum*	48th	0·2	...	...	...	
		49th	0·2	...	...	...	
		50th	0·2	...	...	...	
		51st	0·2	...	...	...	
		54th	0·2	...	...	...	
		65th	0·2	...	...	...	
		66th	0·2	...	...	...	
		67th	0·2	...	...	...	
		70th	0·1	...	...	133rd	Rat inoculated on 132nd day did not become infected.

In Tables VI and XI are given the results of treating donkeys infected with *T. rhodesiense* and *T. equiperdum*. On the whole, the results obtained were unsatisfactory. Possibly owing to the fact that almost every injection gave rise to abscess formation, the drug exerted little influence on the number of parasites in the peripheral blood except in the case of Donkeys 32 and 60 infected with *T. equiperdum*. All the treatment animals became rapidly emaciated, and died sooner than the controls.

TABLE VII.—Giving the results of treating small animals, infected with *T. rhodesiense*, by intravenous injection of a suspension of Antimony trioxide in distilled water.

No.	Animal	Day of treatment	Amount of Sb_2O_3 given, in mg. per 10 g. of body weight	Day on which trypanosomes disappeared from the blood	Day of relapse	Day of death	Remarks
84	Rabbit	17th	0·1	18th	24th	...	
		26th	0·06	26th	...	...	
		29th	0·1	...	29th	29th	Animal died with convulsions at termination of the injection.
85	,, ...	25th	0·15	...	...	25th	,, ,,
87	,, ...	17th	0·1	18th	...	...	
		21st	0·1	...	29th	...	
		26th	0·05	...	...	...	
		36th	0·1	30th	39th	41st	
89	Guinea-pig ...	17th	0·25	18th	39th	...	
93	Rabbit	17th	0·1	18th	...	...	
		21st	0·15	...	...	...	
		26th	0·05	...	...	...	
		29th	0·1	...	...	31st	Trypanosomes did not reappear: animal died negative.
94	,, ...	11th	0·05	12th	...	...	
		13th	0·025	...	...	...	
		15th	0·05	...	17th	21st	
95	,, ...	17th	0·1	18th	...	...	
		21st	0·1	...	...	...	
		26th	0·05	...	...	...	
		36th	0·07	...	51st	...	
96	,, ...	9th	0·02	10th	13th	...	
		13th	0·04	14th	19th	...	
		19th	0·06	20th	...	...	
		24th	0·06	...	27th	...	
		27th	0·2	28th	34th	...	
		34th	0·2	...	...	39th	

Repeated intravenous injections of rabbits infected with *T. rhodesiense* were not attended by good results. As is shown in Table VII relapses were frequent, and trypanosomes only disappeared from the peripheral blood for short periods. Probably this is accounted for by the fact already mentioned that the drug was deposited in the vein at the site of injection, the changes supervening preventing absorption.

TABLE VIII.—Giving the results of treating prophylactically, with Antimony trioxide, guinea-pigs subsequently inoculated with *T. rhodesiense*.

No.	Amount of prophylactic dose of Sb_2O_3 given, in mg. per 10 g. of body weight	Day on which parasites were injected intraperitoneally	Day on which trypanosomes appeared in the peripheral blood	Day of death	Remarks
8	20	Same day	...	...	
		29th	...	40th	Parasites never found; rat inoculated at time of death did not become infected.
9	5	Same day	...	...	
		29th	...	...	
		59th	...	118th	,, ,, ,,
20	4	5th	...	...	
		42nd	...	...	Parasites not found up to 221st day; rats inoculated on the 89th and 160th days after second inoculation of trypanosomes, have not become infected.
22	4	28th	...	49th	Parasites never found; rat inoculated at time of death did not become infected.
23	1	5th	...	...	
		35th	...	...	
		66th	131st	162nd	Animal died with parasites in blood.
24	1	14th	...	...	
		39th	82nd	178th	Only a few parasites seen on 82nd day and never again; rat inoculated from the guinea-pig on the 131st day did not become infected. The guinea-pig died negative.
25	1	21st	...	...	
		57th	69th	...	On the 70th day the animal was given a second dose of the drug equal to 1·5 mg. per 10 g. of body weight. Parasites disappeared two days later and never returned. The animal died negative on the 198th day; a rat inoculated from it on the 131st day did not become infected.

PROPHYLACTIC ACTION OF THE DRUG

A number of normal guinea-pigs and rats were injected intravenously with doses of antimony trioxide ranging from 1 mg. to 20 mg. per 10 grms. of body weight. After varying intervals they were inoculated with *T. rhodesiense* or *T. gambiense.* The object of these experiments was to ascertain the length of time during which a single dose of the drug would protect the animal against infection. The results obtained in the case of infection with *T. rhodesiense* are given in Table VIII, those with *T. gambiense* in Table IX. In the latter the drug had no protective action, a fact which corresponds with the resistance of this strain to treatment.

The incubation period was not increased, and subsequent treatment was without effect. Against *T. rhodesiense* infection there is evidence of distinct prophylactic action. For example, Guinea-pig 20 was inoculated on the 5th and 42nd days after receiving 4 mg. per 10 grms. of body weight of the drug into the muscle. It did not become infected, has increased in weight, and is alive and well 184 days after the second inoculation. Rats injected with its blood on the 89th and 160th days after the second inoculation did not become infected. Again the prolonged incubation period in Guinea-pig 23 is remarkable. This animal which had received 1 mg. per 10 grms. of body weight, was inoculated with trypanosomes on the 5th, 35th and 66th days following the prophylactic dose of the drug. Trypanosomes appeared for the first time on the 131st day, and it died with parasites in its blood on the 162nd day. Another interesting fact is obtained in the case of Guinea-pig 24. This animal had also received 1 mg. of the drug per 10 grms. of body weight intramuscularly. It was inoculated with *T. rhodesiense* on the 14th and 39th days. A few parasites were seen in its blood on the 82nd day; they disappeared next day, and did not reappear. The animal died negative on the 178th day, and a rat inoculated from it on the 131st day did not become infected. No prophylactic action against *T. vivax* was observed in goats, nor against *T. rhodesiense* in donkeys (see Tables X and XI).

Table IX.—Giving the results of treating, prophylactically and subsequently, with Antimony trioxide small animals inoculated with *T. gambiense*.

No.	Animal	Day of treatment after injection of trypanosomes	Amount of Sb_2O_3 given, in mg. per 10 g. of body weight	Day on which trypanosomes appeared in the blood	Day of death	Remarks
55	Guinea-pig ...	Day after	1·0	4th	...	
		11th	1·0	...	...	
		23rd	1·0	...	...	
		37th	1·0	...	68th	Parasites found in blood fairly constantly; animal died with numerous trypanosomes in its blood.
59	„ ...	Same day	1·0	13th	...	
		22nd	1·0	...	...	
		36th	1·0	...	77th	„ „
61	Rat	Same day	1·8	6th	...	
		7th	1·8	...	...	
		17th	1·8	...	...	
		25th	2·5	...	26th	Parasites constantly present in very large numbers.
62	„	Same day	1·6	6th	...	
		7th	1·6	...	...	
		17th	1·6	...	18th	„ „
63	„	Same day	1·6	6th	...	
		7th	1·6	...	9th	„ „
64	„	Same day	1·5	6th	...	
		7th	1·5	...	...	
		17th	1·5	...	...	
		25th	2·0	...	...	
		35th	1·0*	...	...	
		39th	2·0	...	46th	„ „

*Intraperitoneal injection of 1 per cent. suspension in saline solution.

TABLE X.—Giving the results of treatment of goats, infected with *T. vivax*, with Antimony trioxide, prophylactically and subsequently.

No.	Day of treatment	Amount of Sb_2O_3 given, in mg. per 10 g. of body weight	Day on which trypanosomes disappeared from the blood	Day of relapse	Day of death	Remarks
26	14 days before	1·0	...	8th	...	
	9th	0·2	...	...	...	
	14th	0·2	...	...	17th	Trypanosomes constantly present in the blood.
27	21 days before	1·0	...	23rd	...	
	24th	0·05	24th	...	...	
	26th	0·05	...	...	...	
	27th	0·05	...	...	...	
	29th	0·05	...	...	...	
	31st	0·05	...	31st	...	
	32nd	0·05	32nd	...	...	
	33rd	0·05	...	...	...	
	35th	0·05	...	...	...	
	45th	0·05	...	...	...	
	52nd	0·05	...	...	109th	Trypanosomes never found after the 32nd day; animal died negative.
28	23 days before	1·0	...	22nd	...	
	24th	0·05	...	...	...	
	25th	0·05	...	...	...	
	27th	0·05	...	...	...	
	29th	0·05	...	...	...	
	30th	0·05	...	...	...	
	31st	0·05	31st	...	...	
	33rd	0·05	...	...	...	
	35th	0·05	...	...	...	
	43rd	0·05	...	...	...	
	49th	0·05	...	49th	...	
	50th	0·05	50th	...	...	
	51st	0·05	...	...	...	
	52nd	0·05	...	184th	...	
	184th	0·1	185th	...	...	
	187th	0·1	...	...	...	
	193rd	0·1	...	...	201st	Trypanosomes never found after the 185th day; animal died negative.

TABLE XI.—Giving the results of treating with Antimony trioxide, prophylactically and subsequently, donkeys infected with *T. rhodesiense*.

No.	Day of treatment after injection of trypanosomes	Amount of Sb_2O_3 given in grammes.	Day on which trypanosomes appeared in the blood	Day of death	Remarks
35	21 days before	2·0	9th	11th	
36	21 days before	2·0	9th	...	
	34th	0·2*	...	...	*Given in five different places.
	47th	0·2†	...	49th	†Given in two different places. Trypanosomes always present in blood.
65	18 days before	0·8*	8th	56th	*Given in two different places. Trypanosomes almost always found in blood.
66	18 days before	0·8*	8th	...	
	11th	0·1	...	...	
	12th	0·1	...	...	
	13th	0·1	...	...	
	14th	0·1	...	...	
	16th	0·4	...	...	
	18th	0·1	...	...	
	23rd	0·1	...	...	
	25th	0·1	...	...	
	27th	0·1	...	...	
	33rd	0·1	...	...	
	41st	0·2*	...	...	*Given in two different places.
	44th	0·2*	...	...	
	47th	0·4†	...	61st	†Given in four different places. Trypanosomes almost always present in blood.
67	18 days before	0·8*	8th	...	
	11th	0·1	...	...	
	12th	0·1	...	...	
	13th	0·1	...	...	
	14th	0·1	...	...	
	16th	0·4	...	...	
	18th	0·1	...	...	
	23rd	0·1	...	...	
	25th	0·2	...	...	
	27th	0·1	...	...	
	33rd	0·1	...	...	
	40th	0·3*	...	43rd	*Given in two different places. Trypanosomes almost always present in blood.

Except in the case of *T. gambiense* and *T. lewisi* the drug had an extraordinary effect in controlling the infection, so that the number of parasites found in the peripheral blood was usually very small. Frequently, as is shewn in Table XII, the peripheral blood remained free from trypanosomes for long periods.

Table XII.—Giving instances of relapse after long negative periods.

No.	Animal	Strain	No. of days in which peripheral blood was negative. Relapse period	Remarks
78	Guinea-pig	*T. rhodesiense* ...	98	Trypanosomes found by sub-inoculation.
79	,, ...	,, ...	102	
6	,, ...	*T. ugandae*	137	
49	Rat	*T. equiperdum* ...	77	
48	,, ...	*T. congolense* ...	79	
28	Goat	*T. vivax*	134	
60	,, ...	,, ...	101	
23	Guinea-pig	*T. rhodesiense* ...	65	
32	Donkey	*T. equiperdum* ...	106	Sub-inoculated rat became infected.

CONCLUSIONS

1. As regards the amount of antimony trioxide which can be given at a single dose without causing toxic effects, either local or general, it was found that, as is generally the case, a relatively much larger quantity is borne by small than by large animals. At least 1 mg. per 10 grms. of body weight can be administered intramuscularly, or intraperitoneally to rabbits, guinea-pigs, rats and mice without any untoward result. In donkeys and dogs, on the other hand, such a dose frequently caused death, and even much smaller doses were followed by abscesses at the site of inoculation.

2. Although the amount of drug administered can be regulated, we have, unfortunately, no control over the amount or rate of absorption. Post-mortem evidence proves that the proportion absorbed during a period of six months is exceedingly small. Probably this factor explains the somewhat unsatisfactory and contradictory results obtained.

3. In view of the extremely long periods after which relapses have occurred, we must be very cautious in stating that an animal is cured. But as several have remained negative without relapse for over 200 days, and subinoculated animals have not become infected, it appears that a certain number of cures have resulted.

4. Most of the strains used by us, viz., *T. rhodesiense*, *T. brucei*, *T. ugandae*, *T. evansi*, *T. equiperdum*, *T. congolense*, and *T. equinum*, appear to be very susceptible to the drug and trypanosomes disappear from the blood of infected animals within a couple of days of treatment. Our laboratory strains of *T. gambiense* and *T. lewisi* proved to be refractory.

5. Relapses after the blood had been negative for at least 100 days occurred in animals infected with *T. rhodesiense*, *T. ugandae*, *T. vivax* and *T. equiperdum*.

6. The drug exerted a definite prophylactic action when injected into guinea-pigs against subsequent infection with *T. rhodesiense*, but none against our laboratory strain of *T. gambiense*. No prophylactic effect was noticed in goats against *T. vivax* nor in donkeys against *T. rhodesiense*.

In conclusion, we wish to express our indebtedness to Mr. Walter K. Fernie for generously providing the funds for this research.

OBSERVATIONS ON VARIATIONS IN FORM OF MICROFILARIAE FOUND IN MAN

BY

J. E. L. JOHNSTON, M.B., B.S. (LOND.),
D.T.M. & H. (CANTAB.)
WEST AFRICAN MEDICAL STAFF

(*Received for publication* 16 *February*, 1914)

PLATE IV

Of the family *Filariidae*, Braun (1895), the following members producing sheathless embryos have been found in man:—

Sub-family *Filariinae*, Stiles (1907).

1. *Filaria demarquayi*, Manson (1897). The microfilaria shows periodicity, measures 200μ by 5μ, and terminates posteriorly in a finely pointed tail.

2. *Microfilaria philippinensis*, Ashburn and Craig (1906). Possesses a tightly-fitting sheath that can be easily overlooked, hence I include it, for the purposes of this paper; no periodicity; measures 290-335μ.

Bahr suggests that it may possibly be *Microfilaria bancrofti* that has lost its periodicity.

3. *Acanthocheilonema perstans*, Manson (1891). Shows no periodicity; measures 190-210μ, the body tapering slowly in the posterior two-thirds, the tail abruptly rounded.

Shorter forms, measuring 90-100μ, have been described. Stained specimens show nuclear breaks at about (i) 17 per cent., (ii) 24·5 per cent., (iii) 62·5 per cent. of the length.

4. *Dirofilaria immitis*, Leidy (1856). Length 285-295μ, with a tapering posterior extremity. This filaria occurs in the dog, especially in the Far East, and is of exceedingly doubtful occurrence in man.

Sub-family *Onchocercinae*, Leiper (1911).

5. *Onchocerca volvulus*, Leuckart (1893). The microfilaria has so far not been found in the blood. It measures 250-300μ, with a pointed tail.

Whilst I was stationed in Bassa Province, Northern Nigeria, I met with the following case amongst the prisoners:

Male, Ami, about 50 years of age, Ndoma pagan. Complained of pains in the back, apparently lumbago. A few weeks later he showed swelling of both feet and legs, a firm hard oedema, with indefinite pains from the knees downwards.

The myalgic pains in the back recurred, and were but slightly benefited by treatment.

There were no other signs or symptoms, heart and lungs normal, liver and spleen not palpable; no abdominal ascites; no albuminuria.

The stools showed ancylostome ova in scanty numbers. A differential count of the leucocytes showed the following:—

Polymorphonuclears 60 per cent., Eosinophiles 3 per cent., large Mononuclears 3 per cent., small Mononuclears 34 per cent. The haemoglobin content was 60 per cent.

There was, at the time, a curious epidemic of dropsy amongst the prisoners, some presenting a close resemblance to beri-beri, others to ankylostomiasis, and many were found to harbour ancylostomes and other intestinal parasites;* an exact diagnosis in each case was therefore extremely difficult.

In this particular case, anthelmintics, such as thymol, or the eucalyptus, chloroform and castor oil mixture, produced no improvement in the condition.

Further, examination of the blood showed microfilariae of three definite types:—

A. Embryos varying in length from 83-170μ, without sheaths, actively motile, with a tapering posterior extremity ending in a bluntly-pointed tail.

Careful examination of the living blood showed rapid movements, both darting and serpent-like, gradually slowing, so that after twenty-four hours there were but faint flickers of the body, after twenty-eight hours none.

B. Embryos of 230-251μ, resembling the above closely, but having a very sharply-pointed tail.

These are the two types on which I would lay stress. They were very scarce in the blood, and I was only able to obtain blood-films showing 33 in all (all obtained during the day), which I have carefully drawn, and measured by the method of tangents.

---

* A Note on Helminthiasis in Bassa Province, N. Nigeria, by the present writer.—'Lancet,' Sept. 27, 1913.

C. Embryos about 300μ in length, sheathed, apparently *Loa loa.*

I am unable to say whether there was any connection between the presence of types A and B, and the general physical state; *Loa loa* produced no clinical manifestation.

TYPES A AND B. I was unable to discern the central viscus of Manson. Both types presented four very definite nuclear breaks, their positions being shown in Tables I and II, the second portion of each table giving the relative percentage position, considered from the anterior extremity, and taking the central point of each break as the percentage point. (Where the central point lies between two consecutive figures, e.g., 64 and 65, I have taken the lower, 64.)

Type A. Length 83-170μ. The mean positions of the breaks occurred at the following points:—

(i) 23·2 per cent., (ii) 32·9 per cent., (iii) 62·6 per cent., (iv.) 83·5 per cent. (See Table I, p. 76).

Of the microfilariae mentioned as previously described, the only one that would in any way correspond with this is *Acanthocheilonema perstans,* supposing forms to occur varying from 83-170μ. But the forms described are, the normal of 190μ (*circa*), and the small forms of 90-100μ; no mention is made of forms between 100 and 170μ.

The nuclear breaks occur at, roughly, 17 per cent., 24·5 per cent., 62·5 per cent., not at 23·2 per cent., 32·9 per cent., 62·6 per cent., and 83·5 per cent.

Type A shows the following anatomical characteristics: the nuclei are coarse, though not so coarse as Type B; usually three abreast through the greater part of the body. The anterior extremity is devoid of nuclei for a distance of 2 to 4, even 6μ. The first break is very narrow, sometimes merely a finely cut line, but it goes across the whole width of the body. The second is also complete, and is usually about 4μ in length. The third is small, and though wider than the first, seldom crosses far beyond the mid-line; the fourth varies from an almost invisible break to a complete gap of 4 to 5μ.

Figures 1, 2 and 3, Plate IV, illustrate these points.

TABLE I.—Showing the Positions of the Nuclear Breaks in Type A

BLUNT-TAILED

No	L'ngth	A.B.	Breaks in μ				Percentage			
			(1)	(2)	(3)	(4)	1	2	3	4
1. (2)	83	—	—	24–26	—	65–67	—	30	—	80
9. (4)	93	1–2	22	32–35	58–59	76–78	24	35	62	83
8. (3)	94	1–2	22	30–33	57–58	80–81	23	33	62	85
2. (4)	94	—	—	31–35	63–68	80–85	—	35	69	87
8. (2)	95	1–2	23	31–32	55–57	78–80	24	33	59	83
2. (5)	99	—	—	28–31	55–60	76–80	—	29	57	78
8. (4)	102	1–4	25	35–37	64–66	87–90	25	35	63	86
8. (1)	107	1–2	24	32–36	69–70	88–91	22	32	65	83
4. (1)	111	—	23	35–39	72–74	96–98	21	33	66	87
9. (2)	113	1–3	26	37–39	75	93–97	23	34	66	86
8. (5)	115	1–3	26	32–39	75	97–101	23	30	65	86
5. (1)	117	1–4	24	32–36	72–75	97–99	21	29	62	84
9. (6)	118	1–4	29	38–44	76	96–102	24	35	65	84
9. (5)	119	1–4	29	38–44	78	98–102	24	34	66	84
7. (2)	132	1–5	33–34	42–47	77–80	109–115	25	33	59	85
12. (3)	137	1–3	30	40–42	80–86	110–114	22	31	60	82
6. (2*a*)	143	1–7	36–38	56–64	—	120–122	26	42	—	83
9. (3)	148	1–5	35	45–50	94–97	128–130	24	32	63	76
12. (1)	148	1–6	37	51–55	96–99	120–124	25	36	66	82
6. (2*b*)	156	1–4	34–36	48–52	93–97	130–134	22	32	61	85
6. (3)	164	—	34–38	50–54	96–99	140–144	22	32	60	87
2 (2)	165	1–4	38	48–55	95–97	136–145	23	31	58	85
5. (4)	169	1–4	34–37	46–51	95–99	—	21	29	57	—
4. (3)	170	1–4	—	60–64	110–114	137–140	—	36	66	82
						Mean ...	23·2	32·9	62·6	83·5

TABLE II.—Showing the Positions of the Nuclear Breaks in Type B

SHARP-TAILED

No.	L'ngth	A.B.	Breaks in μ				Percentage			
			(1)	(2)	(3)	(4)	1	2	3	4
5. (1*a*)	230	1–6	56–58	72–77	157–160	195–200	24	32	69	86
6. (4)	235	1–6	52–58	72–78	160–164	196–200	22	31	67	84
4. (2)	242	1–6	48–52	72–75	—	192–196	21	30	—	80
5. (2)	245	1–7	56–59	80–84	166–174	204–206	23	33	69	84
4. (4)	246	1–5	55–59	78–82	—	200–205	23	33	—	82
5. (5)	247	1–6	53–56	76–81	169–178	200–205	22	32	70	82
7. (3)	251	1–6	56–61	82–87	171–175	208–211	23	33	69	83
						Mean ...	22·6	32	68·8	83

TABLE III.—

Two specimens probably sheathed

No.	L'ngth	A.B.	Breaks in μ				Percentages				
			(1)	(2)	(3)	(4)	1	2	3	4	5
1. (1)	182	37	57	114	143	156	20	31	63	79	86
5. (3)	185	—	63–67	—	150–154	163–165	—	35	—	82	88

Type B. Length 230-251μ. The nuclear breaks appear at the following points: 22·6 per cent., 32 per cent., 68·8 per cent., 83 per cent. (see. Table II, p. 77); the break at 68·8 per cent. apparently corresponding with the break at about 62·5 per cent. in Type A, or in *A. perstans*. There appears to be no type closely resembling Type B.

The internal anatomy is as follows: The nuclei are usually extremely coarse, averaging about two abreast, except near the posterior extremity, where they are single. The clear anterior extremity is about 5, sometimes even 7μ in length. The first break is considerably wider than in Type A, but is seldom complete; the remaining breaks are relatively small, nor are they often complete.

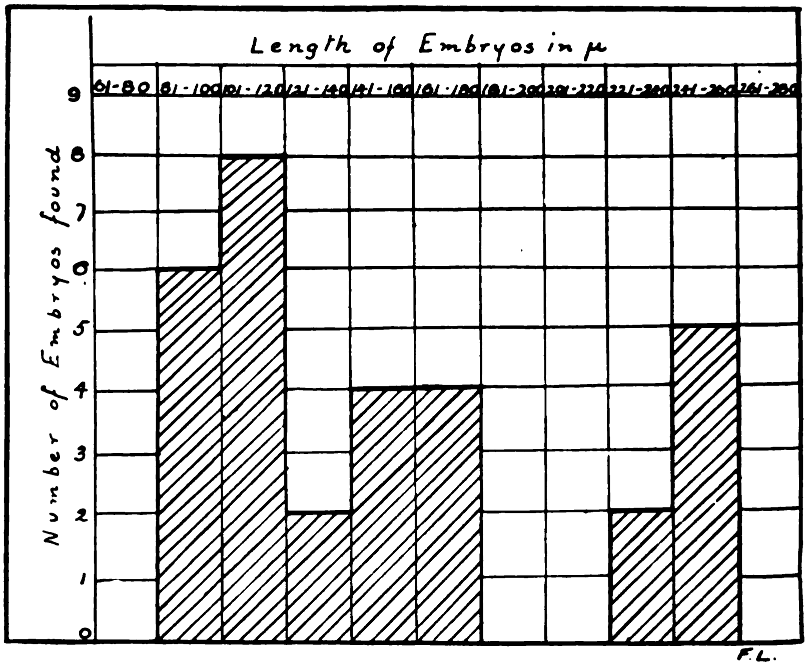


CHART I

Chart I gives a graphic numerical representation of these two types, but I would point out that my material was scanty, and that larger numbers would probably do away with irregularities.

There remained two microfilariae of 182 and 185μ in length, but both appeared to be sheathed. (See Table III, p. 77.)

It is difficult, judging from the embryos alone, to decide whether these two types, A and B, constitute new species.

I had no opportunity of obtaining the adult form, and my supply of the embryonic form was small. One should rather suggest, therefore, that these types might be variations in form of some previously-described microfilaria, such as *Acanthocheilonema perstans*, unless further observations should prove that they are in reality new species.

MEDICAL RESEARCH INSTITUTE,
YABA, SOUTHERN NIGERIA.

DESCRIPTION OF PLATE IV

Magnification, 1,000 diameters in each case.

Figs. 1-4. Various microfilariae found in the blood of a native patient in Bassa Province, Northern Nigeria.

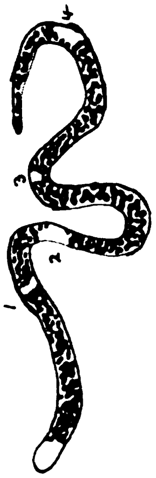


Fig. 1

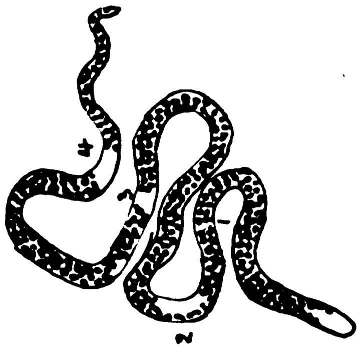


Fig. 2

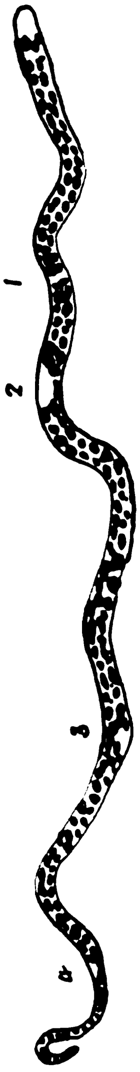


Fig. 3

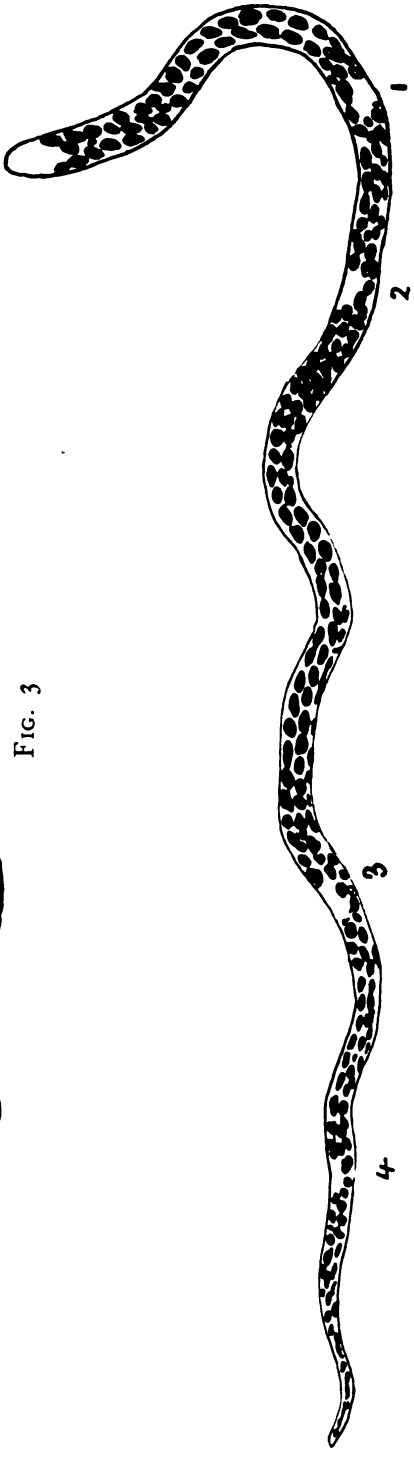


Fig. 4

C. Tinling & Co. Ltd., Imp.

A NOTE UPON THE OCCURRENCE OF A *PLASMODIUM* IN THE BLOOD OF WEST AFRICAN MONKEYS

BY

HARALD SEIDELIN, M.D.

AND

ANDREW CONNAL, M.D.

(Published by permission of the Yellow Fever (West Africa) Commission of the Colonial Office)

(Received for publication 4 *March,* 1914)

Blanchard and Langeron (1913) and Léger and Bouilliez (1913) have recently discussed the possibility of monkeys being in the possession of acquired immunity towards *Plasmodium* infections. The question thus raised is, for various reasons, an important one; the answer obviously would depend upon the results obtained from the examination of the blood of young monkeys in their native countries.

It would probably be difficult for a single observer to collect specimens from a large number of monkeys in a certain district. Hence, knowledge of the prevalence of these blood parasites will probably, to a large extent, have to be acquired through observations on small series of animals, for which reason it may be of interest to record our very limited experience.

Three young monkeys were received from the interior and examined at the Medical Research Institute, Yaba, near Lagos, Southern Nigeria. Two of the monkeys were *Papio sphinx*, and one was a *Cercopithecus mona*. In all three cases the examination of the blood showed the presence of pigment-containing parasites, evidently belonging to the genus *Plasmodium*. The parasites were in all cases very scarce, and the majority of the forms observed were gametocytes. In fact, in one case no schizonts at all were seen, and in the other two cases we found, besides full-grown gametocytes, only a few young ring forms, but no elements in a stage of advanced schizogony. We shall therefore not discuss the question to which species of ***Plasmodium*** the parasites belong, but we wish to mention a few morphological observations.

These 'malarial' parasites differ in various respects from those of the human being. The most striking morphological difference is found in the appearance of the pigment, which in fresh specimens is of a bright yellow colour, instead of the dark brown observed in human blood; in Giemsa-stained specimens the pigment shows a brilliant green colour.

Another peculiarity which should be mentioned consists in the comparatively faint staining of the chromatin, which altogether appears to be of a less compact structure than that of the gametocytes in the human blood. The protoplasm is likewise faintly stained, especially that of the microgametocytes, but also the macrogametocytes appear much less deeply blue than the corresponding forms of the human parasites.

The chief interest of this communication is, we believe, that these three monkeys were the only ones which we received at the time of the observations, and that they were all young individuals. The number is small, but the fact that all three were infected is suggestive. It seems quite possible that native monkeys may show conditions similar to those encountered in native humans in West Africa, where practically all individuals are infected with malaria at an early age, thus acquiring for life a more or less marked immunity. As far as we have observed, none of the monkeys showed any symptom of malaria, but our observations on this particular are not conclusive, as the animals were used for experimental purposes.

It may be added that one of us (Seidelin, 1911) found a *Plasmodium* in the only monkey which he had at his disposal in Yucatán (Mexico), in which case intermittent fever was observed.

REFERENCES

Blanchard, R. et Langeron, M. (1913). Le Paludisme des Macacques. Arch. Parasitol. XV, 4, pp. 529-542.

Léger, M. et Bouilliez, M. (1913). Recherches expérimentales sur '*Plasmodium inui*,' etc. Ann. l'Inst. Pasteur, XXVII, 11, pp. 955-985.

Seidelin, H. (1911). Notes on some blood parasites, etc. Ann. Trop. Med. Paras., V, 4, pp. 501-508.

NOTE ON SCRATCHING BIRDS AND TSETSE-FLY

BY

LLEWELLYN LLOYD

(*Received for publication* 12 *March*, 1914)

From time to time suggestions have been made that various scratching birds might act as a control of *Glossina* by finding and devouring the pupae. The birds which have been mentioned in this connection are the guinea fowl, the Indian jungle fowl and the domestic fowl. Of these the first and the last are well represented in the fly areas of Central Africa, together with three species of partridge. The domestic fowl is kept in some numbers in all the villages in Northern Rhodesia. It remains, however, entirely in the vicinity of the village, among the huts, feeding upon grain accidentally scattered, chaff from the winnowing and insects, especially ants and small Orthoptera. Since tsetse fly are known to avoid villages and certainly do not breed in their immediate neighbourhood, the fowls would have no opportunity of finding the pupae.

Guinea fowl are extremely numerous in Northern Rhodesia, especially in the Luangwa Valley where, in the early morning, hundreds may be seen together on one of the short-grassed plains. An examination of the contents of the crops of ten guinea fowl was made at Nawalia, in the Luangwa Valley. The crop of each was filled with vegetable matter, small bulbs, roots and flower buds. In three only were insects found, and these were in very small numbers. The following insects were observed in them: small Staphyline beetles, coleopterous larvae and lepidopterous larvae. No pupae of any kind were recorded. From this it is clear that the guinea fowl is a vegetable feeder, eating insects when it finds them in its search for vegetable food. In the case of *G. morsitans* the majority of the pupae are placed in positions which would be inaccessible to these birds, though a few could be easily found. Doubtless a few of the pupae are eaten by guinea fowl, but the number must be so small that the bird cannot be considered to act as a control.

THE ORIGIN AND DEVELOPMENT OF GAMETES (CRESCENTS) IN MALIGNANT TERTIAN MALARIA: SOME OBSERVATIONS ON FLAGELLATION, Etc.

BY

DAVID THOMSON, M.B., Ch.B. (Edin.), D.P.H. (Cantab.)
GROCERS' SCHOLAR

(Being Part II of the Report on the Malarial Expedition to Panama, 1912)

(Received for publication 21 *January,* 1914)

Plate V

CONTENTS

PAGE
I. Introduction ... 85
II. Deductions made from Observations on Crescents in the Peripheral Blood ... 86
III. Deductions made from Autopsy Smears of the Inner Organs ... 92
IV. Further Remarks ... 96
V. Some Observations on the Flagellation of the Microgametocytes ... 97
VI. Conclusions and Summary ... 100
References ... 102
Explanation of Plate ... 104

I. INTRODUCTION

The part of this research dealing with the study of crescents in autopsy smears of the inner organs was done in conjunction with Dr. W. M. James, Chief Assistant Physician at the Ancon Hospital, Panama, and to him is due the credit of tracing the various stages of the crescent from its earliest beginnings to the adult form. The observations on the crescents in the peripheral blood were made in the Royal Southern Hospital, Liverpool, and full details have already been published (Thomson, 1911). Dr. James continued the work by studying the development of crescents in the inner organs, and, together, we made further observations on this subject in Panama during October, November and December, 1912. The stained specimens of moist chamber crescents (figs. 31-41) were prepared by James.

II. DEDUCTIONS MADE FROM OBSERVATIONS ON CRESCENTS IN THE PERIPHERAL BLOOD

(*a*) *Each crescent develops from a single ordinary asexual spore of P. falciparum.* Mannaberg (1894) put forward the hypothesis that they were produced by the conjugation of two asexual parasites within a red corpuscle, but the fact that cases of malaria with very numerous asexual parasites as a rule produce fewer crescents than those cases with few parasites, is strongly against this hypothesis. In heavy infections one often finds two parasites in one red cell, whereas in mild infections doubly infected red cells are exceedingly scarce, and yet many crescents may be produced. Some observers (Stephens and Christophers, 1908) suggest that they develop from special asexual spores.

(*b*) *It would appear that crescents develop from the asexual spores, due to a development of some immunity or resistance towards the latter.* Some cases of malaria produce gametes in large numbers, while others produce none at all. Their production appears to be determined by the appearance of conditions unfavourable to the asexual cycle. When the asexual spores find that their environment is becoming unsuitable, they undergo a transformation into a sexual generation, and thereby save themselves from destruction. In this new state they remain passive, awaiting their transference into a more suitable host.

If a special asexual spore were set aside to produce crescents, it would be expected that they would be produced in all cases independently of their environment.

The following eight points are in favour of this immunity hypothesis:—

1. Mild, chronic, and probably partially immune cases of malaria show, as a rule, a crescent-producing power fully ten times greater than the very acute cases. There are, however, a few exceptions to this, since acute cases sometimes produce a very large number of crescents.

2. It would appear that crescents are more likely to be produced in cases of long standing, where a certain amount of immunity has had time to develop.*

---

* Dr. James had a remarkable exception, in which a very large number of crescents appeared in a baby only fourteen days old (Case 2866).

3. Of cases with a history of previous attacks of malaria, 87 % developed crescents, whereas only 46 % of the cases with no history of previous attacks, produced crescents. It is reasonable to suppose that these cases, which had a history of previous attacks, were more immune than the primary cases.

4. Patients from 20 to 68 years of age produced more crescents than those below the age of twenty. This might be attributed to a greater power in adults of developing immunity as compared with the young, growing, patients. Many of the older patients, however, had been in the tropics for a long time, and had had previous attacks of fever. The young patients had not been long in malarial districts, and in most cases it was their first attack of malaria. The greater crescent producing power in older patients may, therefore, have been due to immunity developed from previous attacks.

5. A low percentage of haemoglobin is not so favourable for crescent production as a fairly high percentage. This again might be explained by the supposition that immunity to the asexual parasites is more likely to be successfully developed in cases where the blood standard is fairly healthy.

6. Cases with palpable spleens produced more crescents than those with impalpable spleens. According to Ross (1910) and Surveyor (1910), cases with enlarged spleen are more resistant than those without enlargement.

7. Greater numbers of crescents appeared to be produced in cases where the leucocyte count was high, which may again point to increased immunity.

8. Insufficient dosage of quinine would seem to favour the output of crescents. This may be due to the fact that small doses of quinine abate the disease, and so render the condition more favourable for the development of immunity, or it may be caused by quinine rendering the conditions less suitable for the asexual life of the parasite, so that it is induced to take on the sexual phase. It is probably correct to say that small doses of quinine, such as are given prophylactically, tend to produce crescent carriers, without curing the disease.

(*c*) *The crescents develop somewhere in the internal organs, and when completely developed, they appear suddenly in the peripheral blood.* Undeveloped crescents practically never appear in the peripheral circulation. Here again, however, there was one remarkable exception in Dr. James' case (2866), in which all stages of crescents were found in the peripheral blood. (Plate V, figs. 7-14.)

(*d*) *A period of ten days elapses between the appearance in the peripheral blood of the asexual spores, and the crescents which are produced from these spores.* In other words, the crescents take ten days to reach maturity in the inner organs before they appear in the peripheral blood. As a general rule, the crescents appear in the peripheral blood on the fifth day after the attack of fever, and increase in number for four or five days more, so that they are most numerous on about the tenth day after the height of the fever. Those crescents appearing on the fifth day after the paroxysm, correspond to asexual parasites existing in the blood five days previous to this paroxysm. Asexual parasites can exist in the blood in numbers as great as 2,000 per c.mm., and more rarely 10,000 per c.mm. without producing any temperature reaction. They gradually increase in number by sporulation till they are numerous enough to cause a paroxysm of fever. The numbers may then fall spontaneously or by quinine treatment, so that only a single paroxysm results. It is by the study of such single paroxysms that the time required for the appearance in the peripheral blood of the corresponding crescents can be best determined. In such cases, the graphs representing the numbers of asexual parasites and crescents show a striking similarity, the points in the crescent graphs occurring on the tenth day after the corresponding points in the graphs of the asexual parasites. See Charts in previous paper, Thomson (1911).

One case was extremely interesting, as the patient had nine successive daily paroxysms of fever, four paroxysms in the four days before admission, and five paroxysms on the next five days. Corresponding to these nine fever paroxysms or sporulations, there were nine outbursts of crescents into the peripheral circulation, each occurring on the tenth day after the corresponding fever paroxysm. The asexual parasites were rapidly destroyed by quinine after the ninth paroxysm of fever, and a corresponding diminution in the production of crescents is evident ten days later.

Another case, Thomson (1912), showed a tertian outburst of crescents into the peripheral circulation following upon tertian asexual sporulations. The first outburst of crescents occurred five days after admission, and would correspond to a fever paroxysm occurring five days before admission. The patient had had fever for several days before admission. On the tenth day after the administration of quinine, the crescents showed a more or less decided fall in number.

(*e*) *There is evidence to show that the duration of life of a crescent cannot be more than twenty days.* During the first ten days of this period they are developing somewhere, but not in the peripheral blood. They then appear in the peripheral blood. The majority of them perish in the peripheral blood in a very few days. This must be so, for if they lived for say four days or longer, then the summit of the crescent curve could not be a sharp point as it always is, especially when the numbers are great. In certain cases the number of crescents remains high for some days, the graph resembling a kind of plateau containing several sharp peaks. See Charts, Thomson (1911). The explanation of this plateau and apparent persistence of crescents is simple, for if no quinine is given the asexual parasites remain alive, even though there is no fever to indicate their presence, and help in producing new crescents. The source of crescents is not cut off, so that the supply is replenished by new broods of crescents, appearing every day or on alternate days or irregularly, according as the fever or asexual sporulation occurs every day, on alternate days, or irregularly.

The sharp peaks on the plateau show that the crescents are dying rapidly, but that their numbers are replenished by fresh broods coming into the circulation.

In no case is there a plateau formed when quinine has been given in doses of thirty grains daily ten days previous to the height of the crescent curve. If a plateau be formed, and quinine is then administered in large doses, its effect will not be manifested for about ten days, because although it very quickly reduces the source of supply, yet those crescents which commenced to develop during the previous ten days are not affected, but continue to appear in the peripheral blood, replenishing the loss. Hence, though quinine makes the crescents disappear from the peripheral blood more quickly than they would otherwise do without its administration, yet its effect is not due to any direct destructive action in the crescents themselves. It is due indirectly to the destruction of the asexual spores from which the crescents are developed. The length of time that crescents will remain in the peripheral blood, therefore, depends upon the persistence of the asexual parasites. If immunity develops so strongly that the asexual parasites disappear, or if they are destroyed by quinine, then the crescents also will disappear in due course.* In cases where immunity remains, but is only sufficient to keep the number of asexual parasites in check, crescents may continue almost indefinitely. Crescents have been observed to continue in the peripheral blood for eight weeks (Surveyor, 1910). Many observers have noticed this long continuance of crescents, which has led to the fallacious belief that the individual crescents persist for a long time in the peripheral blood. The charts already referred to, show that the majority of crescents must die in the peripheral blood in a very few days. The sharpness of the peaks in the crescent graphs shows that the majority of them must perish in the peripheral blood in one or two days. Observations in many cases of malaria with crescents, 50 or more, show that crescents may be reduced to numbers below the detectable limit in the peripheral blood in less than 18 days by the administration of 20 to 30 grains of quinine daily. This dosage of quinine reduces the number of asexual parasites to below the detectable limit in five days, so that the source of supply is cut off in five days. Those crescents produced from the asexual parasites on the fifth day of quinine will appear in the peripheral blood ten days later, that is, on the 15th day after giving quinine. Three days more have to be accounted for. This may either be the duration of life of a crescent in the peripheral blood, or it may represent crescents developed from surviving asexual parasites after five days' administration of quinine.

In placing the duration of life of a crescent at 20 days, the greatest maximum is, therefore, given, viz., ten days' growth in the inner organs and ten days' life in the peripheral blood. It would probably be more accurate to place this space of life as ten days in the inner organs and one to three days in the peripheral blood.

---

* Cases are frequently met with in which the crescents quickly disappear spontaneously without quinine treatment. This is probably due either to the spontaneous disappearance of the asexual parasites or due to the fact that the asexual parasites cease to form crescents on account of conditions being favourable for the development of the former. In other words, crescents may disappear spontaneously due to a very high or a very low immunity to the asexual parasites.

The graphs of the asexual parasite resemble very closely indeed the graphs of the crescents, the latter occurring ten days later than the former. The rise in both curves is rapid, and the fall in both is at first rapid, then as the numbers become fewer, the fall tails away very gradually till none are left. This alone is sufficient to show that the lifetime of a crescent after its ten days of growth to maturity is very short, for since quinine rapidly destroys the asexual source, and not the crescents themselves, then the crescents would necessarily accumulate, and show a death-rate curve entirely different from that of their producers.

These, then, are the conclusions regarding the source, growth and life of crescents deduced from enumerative observations on the peripheral blood alone. These observations were made by means of a special pipette, Thomson (1911), which enables one to estimate with considerable accuracy and quickness, the number of parasites per c.mm. contained in the peripheral blood; and it should be noted that the most valuable information thus obtained was got, not from single daily observations, but from enumerations made several times daily over a continuous period of some weeks.

(*f*) There is no evidence sufficient or definite enough to support the hypothesis that crescents are able by a process of parthenogenesis to revert back into the asexual phase. The forms described by Schaudinn as parthenogenetic were most probably either atypical sporulation forms due to quinine (Pl. V, figs. 42-45), or else they were partially developed crescents which had found their way into the peripheral circulation (figs. 10 and 11). These spherical, partially developed crescents are probably also the form of parasite described by Craig (1906) as conjugation forms.

(*g*) In all our observations we have found no evidence that male crescents flagellate while in the circulating peripheral blood, thereby fertilising the female crescents, as propounded by Mary Rowley-Lawson (1911).

(*h*) We have found no evidence, therefore, that relapses in malaria are caused by parthenogenesis of crescents, but believe that relapses are entirely due to resistant forms of the asexual phase which have escaped the action of quinine (Ross and Thomson, 1910). This subject has already been thoroughly discussed, James (1913). He believes with Bignami (1912) that the asexual parasites may develop resistance to quinine just as trypanosomes do towards the arsenic preparations, and that these resistant forms by reproducing bring about the relapse.

III. DEDUCTIONS MADE FROM AUTOPSY SMEARS OF THE INNER ORGANS

The total number of cases in which autopsy smears were examined was 194. The general analysis of these cases was as follows:—

	CASES.
Parasites on admission, and parasites at autopsy	33
Parasites on admission, and pigment only at autopsy ...	10
Parasites on admission, and negative at autopsy	11
No record of blood examination on admission, and parasites at autopsy	5
No record of blood examination on admission, and pigment at autopsy	3
No parasites in blood on admission, and parasites at autopsy	18
No parasites in blood on admission, and pigment at autopsy	31
Negative for malaria on admission, and negative at autopsy	83
Total	194

Obviously the only cases from which we were able to draw any definite conclusions regarding the development of crescents were those 33 cases (all malignant tertian) which showed parasites in the peripheral blood on admission, and parasites in the autopsy smears. Information regarding the persistence of crescents during quinine treatment was obtained from some of the other cases. In order to analyse the first 33 cases properly, it was necessary for us to be able to recognise the various stages of crescents from the youngest to the adult forms. I believe that we managed this successfully, and we have depicted them in Plate V (figs. 7-14) and (figs. 22-30). The various stages in the development of the asexual parasites are shown (figs. 1-6), and (figs. 16-21). It will be noticed that the initial stages of the crescent before it takes on its characteristic shape, resemble to some extent the initial stages of the asexual parasite. To distinguish between them, the staining should be as perfect and as deep as possible. The best results were obtained by staining with Hasting's or Wright's stain in the usual way, and then, further staining for 24 hours with Giemsa, and finally clearing away any stain deposit by rinsing the slide rapidly in orange-G.-Tannin. Good results can be obtained, however, by the ordinary method of Giemsa staining. In all its stages, the crescent differs from the asexual parasite in the staining of the protoplasm. The protoplasm of the latter stains a definite blue, whereas the crescent in all its stages stains a greyish blue or steel grey, with sometimes a yellowish tinge. The colour is also fainter than in the asexual parasite, a very good contrast is seen in fig. 6, in which is depicted the two forms of the parasite lying side by side. This happy coincidence was found in the peripheral blood of the remarkable case, 2866, already mentioned (p. 88). There are, however, further differences. The chromatin of the crescent stains more faintly than that of the asexual parasite, and appears to be made up of small granules, and it never segments into separate masses as occurs in the segmenting forms of the latter. Probably the greatest distinguishing feature between the asexual parasite and the crescent, however, is the distribution of the pigment. In the former, the pigment is practically always in one dense definite mass, at first placed eccentrically, later becoming more central. In all stages of the crescent the pigment is always definitely scattered, except in the adult form of the female crescent, where it is more or less concentrated. Finally, another important distinguishing feature is the shape. Even at a very early stage the crescent may take on the typical crescent shape so characteristic of the adult forms; more often, however, the young forms are round or oval, and when about three-quarters grown they become more oval, and then elongate, finally becoming crescentic. The youngest form of the crescent may be distinguished from the youngest asexual form, from the fact that it is heavily pigmented for its size, whereas no pigment is found in the youngest asexual parasite.

Most of these observations on the morphology of the developing crescent were made in the remarkable case, 2866,—a negro baby, age three weeks, which died shortly after admission to Ancon

Hospital, Panama. It had had fever during fourteen days previous to admission, according to the mother's statement. Numerous anophelines were found in the house, and it had, probably, been infected soon after birth. This case is a remarkable one, because it exhibited in the peripheral blood, not only all stages of the asexual form of the malignant tertian parasite, but, in addition, all stages of the developing gametes or crescents. It is not very common to find adult asexual forms of the malignant tertian parasite in the peripheral blood, and it is certainly most exceptional to find developing crescents. The next problem was that of determining the time required for the development of the crescent from the youngest to the adult form. It was already deduced from peripheral blood observations that the period of development was ten days in the inner organs. Among the 33 cases containing parasites at autopsy, seventeen cases showed crescents at some stage of development, and by a careful analysis of these we were able to find confirmation of the above deduction. On the assumption that the crescents develop from the youngest form of the asexual parasite, and further assuming that large doses of quinine quickly kill off the asexual, but not the sexual forms, then a case treated for ten days with quinine should show only adult crescents in autopsy smears. Cases treated for five days should show only half grown to adult forms of the crescent. Cases treated for about three days should show gametes from about one quarter-grown upwards, and untreated cases should show the asexual forms from which the gametes develop, and at the same time all stages of developing gametes or crescents. By carefully noting the amount of quinine treatment given before death and the various stages of crescents found at autopsy, we were able to obtain strong confirmatory evidence that our assumptions were correct. The details of these seventeen cases were as follows:—

Four cases were untreated with quinine, having died almost immediately after admission to hospital.

1. Case 2653. Autopsy smears of the spleen and bone marrow showed many sporulating asexual parasites, and a few very young developing crescents, but no adult forms.

2. Case 2866. The spleen and marrow smears showed all stages of the asexual cycle and all stages of crescents.

3. Case C. S., treated by Christian Scientist. The spleen and marrow showed very numerous sporulating asexual parasites, and a few crescents from the very youngest to half-grown forms.

4. Case C. The spleen and marrow showed numerous young asexual parasites and all stages of developing crescents as well as adult forms.

Three cases died after one day's treatment with quinine.

5. Case 2873. Received 65 grains of quinine. Autopsy smears showed scanty segmenting asexual forms, and a few crescents in all stages of development.

6. Case 2. Received 100 grains of quinine, and showed numerous asexual parasites and crescents of all stages at autopsy.

7. Case 2707. Received 30 grains quinine, and showed all stages of asexual and sexual parasites at autopsy.

Three cases died after two days' quinine treatment.

8. Case 1. Received 60 grains of quinine per day, total 120 grains. The spleen and marrow showed many young asexual forms and very scanty crescents from the youngest upwards.

9. Case 2. Received 120 grains of quinine in 26 hours, all forms of asexual parasites and all stages of crescents except the very youngest were found at autopsy.

10. Case 3. Received 100 grains of quinine in 26 hours. The autopsy showed all stages of asexual parasites and all stages of crescents but the youngest.

One case died after three days' quinine treatment.

11. This case had 125 grains of quinine during these three days. Autopsy showed all stages of asexual parasites, and also young crescents up to half-grown forms. It should be noted, however, that the young crescents were accompanied by an asexual generation in full development.

One case died after four days' quinine treatment.

12. This case received 45 grains of quinine daily for four days. The autopsy showed degenerating half-grown asexual parasites, and only half-grown to adult crescents.

Two patients died after five days of quinine treatment, 30 grains daily.

13. Case 1. The autopsy showed very scanty asexual parasites and numerous crescents from half-grown to adult forms.

14. Case 2. Autopsy showed no asexual parasites, and only half-grown to adult crescents.

The remaining three cases 15, 16 and 17 died after 10 days' quinine treatment of an intercurrent disease.

15. Autopsy showed no asexual parasites, and only three-quarters to adult crescents. (Quinine treatment 45 grains daily).

16. Autopsy showed no asexual parasites, and only one crescent (adult) was found. (Quinine treatment 45 grains daily).

17. Autopsy showed no asexual parasites, and only three-quarters to adult crescents. (Quinine treatment 15 grains daily).

From these cases, it would seem to us that the evidence goes to support the deduction that quinine cuts off the asexual source of supply of crescents, but that it does not destroy the crescents themselves at any stage of development, and that it would appear that the period required for their full development in the spleen and marrow is about ten days.

IV. FURTHER REMARKS

Some further observations regarding the persistence of crescents and pigment were brought forth in this research. In two cases adult crescents were found in autopsy smears after ten and eleven days' treatment with quinine (45 grains daily). And in seven cases malarial pigment was found in the spleen and marrow smears after 10, 11, 12, 13, 21, 24, 32 and 47 days' treatment with quinine (30 grains daily). In a few cases showing parasites on admission, no pigment was found in the autopsy smears. These as a rule had a long course of quinine (45 grains daily) for 25 to 40 days.

It was observed by Dr. James that some cases of blackwater fever showed very little evidence of pigment in the autopsy smears, even although numerous parasites had been present in the peripheral blood before the attack of blackwater, and only a few days before death. It is difficult to account for this. While working on the cultivation of malaria in vitro, it was noted by Dr. J. G. Thomson and myself that the malarial pigment persists in the culture tubes for months, and, in fact, it would seem to persist in the tubes indefinitely.

Regarding the problem of parthenogenesis, we have found no evidence that this occurs in crescents, and it is quite probable that the forms described by the few who claim to have seen parthenogenesis, were either developing gametes, or else they were atypical sporulating schizonts, vide James (1913). In anaemic patients, and where a considerable amount of quinine has been given, the sporulation is often atypical (figs. 42-45). These forms are, in fact, unhealthy or degenerating parasites. The staining of the protoplasm is very faint, and the number of spores produced is much less than the normal number. Forms exactly similar are seen in cultures after four or five days. Sometimes in the same culture tube, the asexual parasite may pass through two, three, and even four generations, but the latter generations show signs of decay in that the number of spores produced is much less, only 6 to 10 being developed instead of the usual 24 to 32. The staining of the protoplasm also became very faint, and often it takes on a faint pinkish colour instead of the usual blue. The more advanced degenerated sporulation forms show only a mass of granular pinkish chromatin.

Finally, we have found no evidence in autopsy smears of the resting stages of the malarial parasite described by Craig, and it has been pointed out by James (1913), that these were probably developing gametes, such as depicted in figs. 10, 11, 27 and 28. In a few autopsy spleen smears we found bodies as depicted in figs. 46, 47 and 48. These were rather scarce, and we have been unable to determine what they may be.

V. SOME OBSERVATIONS ON THE FLAGELLATION OF THE MICROGAMETOCYTES

In Panama, flagellation occurred as early as four minutes after the blood was drawn. In Liverpool, the crescents became spherical in about four minutes, but they seldom flagellated until about twenty minutes after the withdrawal of the blood.

The time of flagellation varies in the same blood, some may flagellate in twenty minutes, some after fifty minutes. Whether the time depends on the temperature or not, I am unable to state. Sir Ronald Ross has been able to observe flagellation occurring even when the slide was kept on ice. I am inclined to believe that the cause of flagellation is osmosis. The crescents become spherical by the passage of fluid into them, and this in turn gives the necessary stimulus for the flagellation to occur. It is generally admitted that breathing on the drawn blood is a good practice if one wishes to get successful flagellation, and it is even better still to add a little water to the blood. Most probably this addition of moisture or water makes osmosis more rapid. In normal blood in the body the osmotic pressure within and without the corpuscles must be about equal, but when the blood is withdrawn, coagulation occurs in about four minutes, and certain lime salts are deposited. The osmotic pressure in the coagulating blood fluid must, therefore, undergo a change, and a flow will take place into the corpuscle and into the body of the crescent, causing it to become spherical. I carried out a few experiments with the intention of bringing support to this hypothesis, and found that if a hypertonic saline solution were added to the drawn blood, the crescents did not become spherical, and they did not flagellate. When hypertonic saline solutions are added to blood, the blood corpuscles tend to shrink and become distorted, due most likely to a flow of fluid from the inside of the corpuscle into the more powerfully-concentrated fluid without. It has been observed by J. G. Thomson and myself that crescents do not become spherical in malaria cultures made after Bass's method. This is probably due to the same cause. In the cultures the blood is defibrinated, and glucose is added, but whether the persistence of the crescent shape is due to the defibrination, or due to the addition of glucose, I am unable to state. In a previous paper I pointed out that flagellation of crescents occurs in the blood from patients who have been subjected to a long and continuous quinine treatment, which shows that quinine does not readily destroy the vitality of crescents. When crescents are studied in wet films under the coverslip, it is noticed that polar bodies, usually two, appear on the margins of the spherical forms, later, if the crescent be a male, exceedingly active and struggling movements can be seen within the body of the parasite, and later, the flagella are extruded. These lash about with great activity and rapidity, and finally get free from the spherical body. Often they do not manage to get free, and they can be observed in rapid movement for a long time, even an hour or more, when the movement gradually gets more sluggish and they apparently die. In stained preparations it would appear that a considerable amount of chromatin is extruded (figs. 31-36), and it is highly probable that this extruded chromatin forms the polar bodies observed in the wet film. In figs. 31 to 34 are depicted what appears to me to be female crescents which have become spherical. They show extruded chromatin, and also a circular pinkish area within the protoplasm. This circular pinkish area contains a centrally-placed dark chromatin dot.

In contrast to these, the male crescents which have flagellated (figs. 35, 36, 40 and 41) show a shrunken body, with extruded chromatin and also several flagella, which appear to arise each from a dot of chromatin within the body of the parasite. Figs. 38 and 39 show male spherical crescents which have not yet flagellated. The latter figure shows what appears to be the coiled-up flagella within the body of the crescent. The flagellum is often extruded in the form of a loop, and later, one end of the loop gets free; sometimes two flagella lie together as one thick flagellum. The number of flagella set free by each microgametocyte is a disputed point. Mary Rowley Lawson (1911) seemed to believe that only one flagellum came from each, but I think that there can be little doubt that the usual number is from four to eight, and fig. 41 would appear to show that even ten can be extruded from one parasite. Whether the number varies or whether it is always the same, I am unable to state. Mary Rowley Lawson also believed that flagellation and fertilisation in the gametocytes occurred in the circulating blood of the human patients. I have been unable to find any supporting evidence of this hypothesis. Spherical crescents are never seen in stained preparations of the circulating blood, provided the smears are dried quickly. I have never had the good fortune to see a free flagellum penetrate into the macrogametocyte. Fig. 37 represents a free flagellum found in a stained moist-chamber-preparation.

Before concluding, I wish to thank Dr. W. M. James for his most valuable assistance, and for his kindness in placing all his material at my disposal. My gratitude is also due to Dr. Henry Carter, and the American Medical Staff in general on the Panama Canal Zone.

VI. CONCLUSIONS AND SUMMARY

DEDUCTIONS FROM PERIPHERAL BLOOD OBSERVATIONS ON CRESCENTS

1. Crescents are produced from ordinary asexual spores of *P. falciparum*, due to a development of immunity towards the latter.

2. They develop somewhere in the internal organs, and then appear suddenly in the peripheral blood.

3. The period required for their development is about ten days.

4. Crescents do not generally live more than a few days in the peripheral blood.

5. Crescents may be present in the peripheral blood during periods as long as eight weeks, not because the individual crescents survive for that time, but because their numbers are constantly replenished from surviving asexual forms.

6. Fresh broods of crescents come into the circulating blood daily, or every other day, or irregularly, according as the asexual sporulations occurring ten days before, were quotidian, tertian, or irregular.

7. Quinine has no direct destructive action on crescents, either during their development or afterwards, but it destroys the asexual source of supply.

8. Quinine in doses of 20 grains daily reduces the crescents to numbers less than one per cubic millimetre of blood within three weeks.

DEDUCTIONS MADE FROM AUTOPSY SMEARS OF THE INNER ORGANS

9. The crescents take about ten days to develop into the adult state in the inner organs.

10. They develop chiefly in the bone marrow and in the spleen.

11. The protoplasm of the crescent in all stages of development stains a faint greyish blue often with a yellow tinge, in contrast to the deeper blue of the asexual schizonts.

The chromatin of the crescents stains fainter, is less abundant and more finely granular than that of the schizonts, and it never breaks up into masses as occurs in the segmenting schizonts.

The pigment in the developing crescents appears early even in the youngest forms, and it remains scattered until the adult stage is reached, whereas in the schizont the pigment in all stages is more or less concentrated into one mass.

The shape of the developing microgametocyte is often spherical. Three-quarters grown forms may even be spherical. Sometimes, however, crescentic and oval-shaped forms may be found very early in development.

12. Adult crescents have been found in autopsy smears of the spleen and bone marrow, after eleven days' quinine treatment (45 grains daily).

13. Malarial pigment has been found in spleen and marrow smears after 47 days' quinine treatment (30 grains daily).

14. No evidence was obtained of parthenogenesis in crescents.

OBSERVATIONS ON THE FLAGELLATION, &C.

15. Crescents become spherical in about four minutes after the blood is drawn, which appears to coincide with the time of coagulation.

16. Crescents may flagellate in four minutes after the blood is drawn, but some may not flagellate till after fifty minutes.

17. The crescents in patients subjected to thorough and continuous quinine treatment are able to flagellate.

18. The spherulation of crescents is probably due to osmosis, which in turn probably stimulates the actual flagellation.

19. The polar bodies seen in crescents which have become spherical are extruded chromatin.

20. The microgametocytes are capable of extruding 4, 6, 8 or 10 flagella (microgametes).

21. There is no reason to believe that crescents ever flagellate in the circulating blood of a patient.

REFERENCES

BIGNAMI (AMICO) (1913). Concerning the Pathogenesis of Relapses in Malarial Fevers. [Translated by W. M. James.] Southern Med. Journ., 1913, February, Vol. VI, No. 2, pp. 79-88.

CRAIG, C. (1906). Observations upon Malaria, Latent Infection in Natives of the Philippine Islands, Intracorpuscular Conjugation. Philippine Journ. of Science, 1906, I, p. 523.

——— (1906). Intracorpuscular Conjugation in the Malarial plasmodium and its Significance. American Medicine, 1906, X, No. 24, pp. 982-986; and No. 25, p. 1029.

JAMES, W. M. (1913). Notes on the Etiology of Relapse in Malarial Infections. Journ. Infectious Diseases, Vol. XII, No. 3, May, 1913, pp. 277-325.

LAWSON, MARY ROWLEY (1911). The Aestivo-Autumnal Parasite; its Sexual Cycle in the Circulating Blood of Man, with a Description of the Morphological and Biological Characteristics of the Parasite. Journ. Experimental Medicine, Vol. XIII, 1911, pp. 263-289.

MANNABERG (1894). The Parasites of Malarial Fever. Marchiafava and Bignami and Mannaberg, New Sydenham Society, 1894 p. 289.

ROSS, R. (1910). Prevention of Malaria, p. 129. A. Celli, p. 406.

ROSS, R., and THOMSON, D. (1910). Some Enumerative Studies on Malarial Fever. Annals Trop. Med. and Parasit., Vol. IV, p. 267.

STEPHENS and CHRISTOPHERS (1908). The Practical Study of Malaria and other Blood Parasites, p. 52.

SURVEYOR (1910). Some Observations on Malaria in relation to Splenic Enlargement and the Treatment of the Crescentic Stage. Annals Trop. Med. and Parasit., Vol. IV, No. 3.

THOMSON, D. (1911). A Research into the Production, Life and Death of Crescents in Malignant Tertian Malaria, etc. Annals Trop. Med. and Parasit., April, 1911, Vol. V, No. 1, pp. 57-82.

——— (1911). A New Blood-counting Pipette for Estimating the Number of Leucocytes and Blood Parasites per cubic millimetre. Annals Trop. Med. and Parasit., Vol. V, pp. 471-478.

——— (1912). Further Observations on the Variations in the Number of Leucocytes and Crescents in Malaria. Annals Trop. Med. and Parasit., Vol. VI, pp. 215-221.

——— (1912). The Destruction of Crescents, etc. Annals Trop. Med. and Parasit., Vol. VI, pp. 223-230.

EXPLANATION OF PLATE V

All figures were drawn from stained preparations.
Magnification, 1,500 diameters.

Figs. 1 to 6. Show various stages of the malignant tertian asexual parasite found in the peripheral blood of the case 2866.

Fig. 6. Shows a segmenting schizont on the left and a three-quarters grown crescent on the right. These two were found lying together as painted.

Figs. 7 to 14. From the peripheral blood of case 2866, represent the various stages in the development of the gamete or crescent.

Figs. 15 to 21. From spleen smears of case 2866, show all stages in the development of the asexual phase of the malignant tertian parasite. Fig. 15 represents a large spleen cell which has ingested four young parasites.

Figs. 22 to 30. From spleen smears of case 2866, show successive stages in the development of the gamete or crescent.

Figs. 31 to 34. From moist-chamber-preparations, made by Dr. James, represent female crescents which have become spherical (macrogametocytes).

Figs. 35, 36, 40 and 41, represent flagellating crescents (microgametocytes).

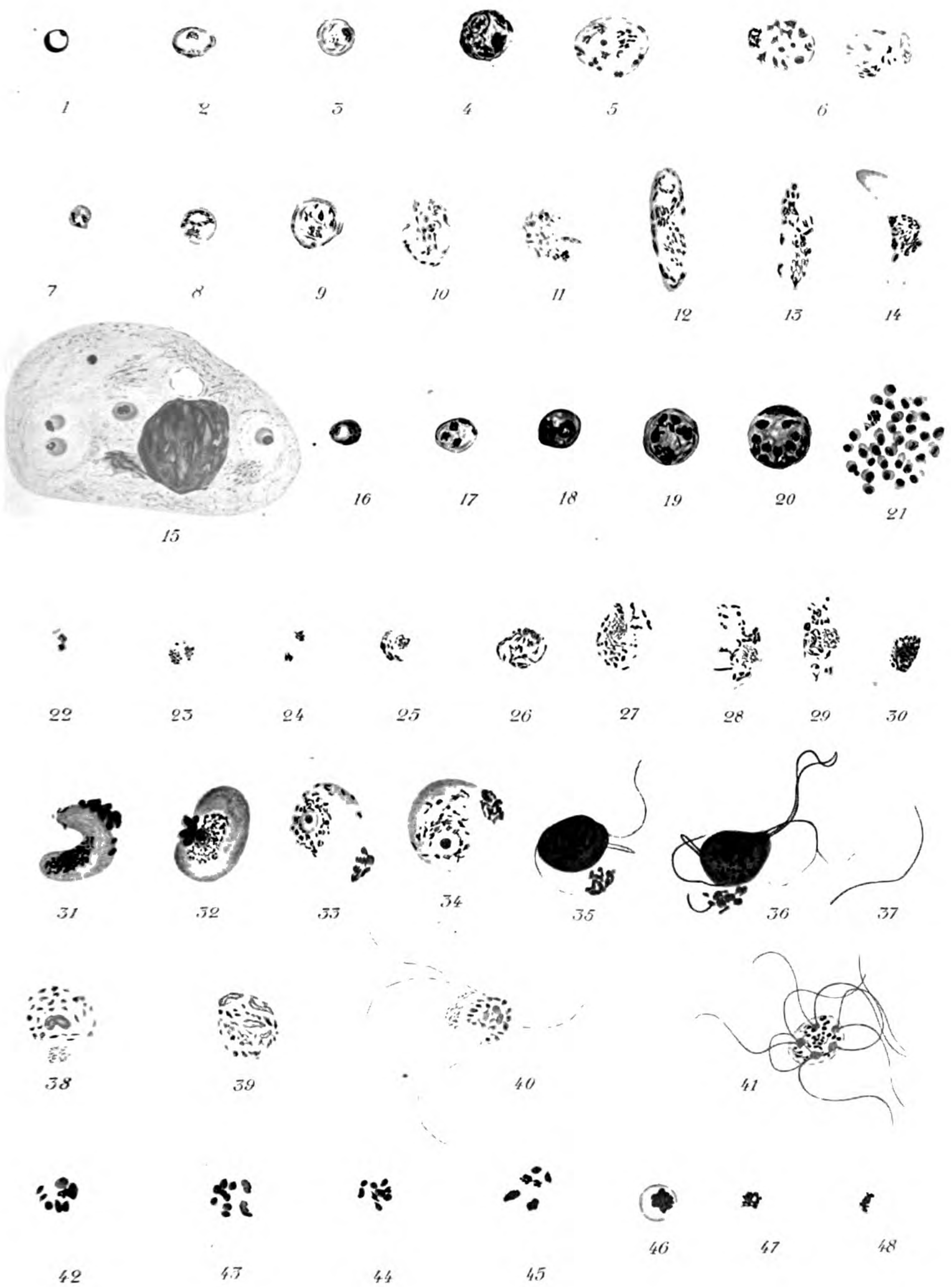
Fig. 37. Shows a free flagellum (microgamete).

Figs. 38 and 39. Represent microgametocytes which have not yet flagellated. Fig. 39 shows the coiled-up flagella.

Figs. 42 to 45. From a smear of the placenta, show atypical sporulation of the malignant tertian parasite due to the action of quinine administration.

Figs. 46 to 48. From autopsy spleen smears, case 3295, represent bodies of undetermined nature. They do not appear to be malarial in origin.

PLATE V



D. Thomson pinx.

[illegible]

SECOND REPORT ON THE CESTODA AND ACANTHOCEPHALA COLLECTED IN QUEENSLAND

BY

T. HARVEY JOHNSTON, M.A., D.Sc., F.L.S.
BIOLOGY DEPARTMENT, UNIVERSITY, BRISBANE

(From the Australian Institute of Tropical Medicine and the Biology Department, University, Brisbane)

(Received for publication 23 *March,* 1914)

PLATE VI

Absence from Australia has prevented an examination of all the material kindly forwarded to me during 1912, by Dr. A. Breinl, the Director of the Australian Institute of Tropical Medicine. Consequently this report is much shorter than it would otherwise have been.

Of the cestodes dealt with, two are regarded as belonging to new species and another to one already known, while four are larval forms. The echinorhynchs have been only cursorily examined as yet.

LIST OF HOSTS AND PARASITES REFERRED TO

Host	Parasite
BIRDS :—	
Sphecotheres maxillaris	*Davainea sphecotheridis*, n. sp.
Grallina picata	*Echinorhynchus*, sp.
Notophoyx novae hollandiae	*Bancroftiella glandularis*, Fuhrm.
Numenius cyanopus	*Echinorhynchus*, sp.
Lobivanellus lobatus	*Gyrocoelia*, sp.

REPTILES :—	
Chlamydosaurus kingii	*Sparganum*, sp. (immature).
Lialis burtonii	*Acanthotaenia* (*Ophiotaenia*) *striata*, n. sp.
Varanus varius	*Acanthotaenia*, sp (immature).
Python variegatus	*Acanthotaenia*, sp. indeterm.
FISH :—	
Seriola lalandi	*Tetrarhynchus*, sp. (immature).
Chrysophrys hasta	*Tetrarhynchus*, sp. (immature).

CESTODA

Davainea sphecotheridis, n. sp.

PLATE VI, FIGS. 5-7

From the fig-bird, *Sphecotheres maxillaris.* In addition to the specimens forwarded by Dr. Breinl, some collected by Dr. T. L. Bancroft at Eidsvold, on the Burnett River, Queensland, were also examined. They all appear to belong to a new species of *Davainea.* The parasite reaches a length of over ten centimetres, its maximum breadth being about 2 mm.

The rounded scolex is sharply marked off from the narrow, unsegmented neck. Its cuticle is ornamented with numerous very delicate spines, which are stronger and more thorn-like around the base of the rostellum. The breadth of the organ is about ·36 mm., the diameter of the prominent strongly muscular suckers being ·14 mm. The latter are deep cups whose edges are armed with abundant closely-set hooklets, arranged in about a dozen alternating rows, the outermost series containing the smallest, while the largest and longest (·01 mm. in length) occur along the margin of each acetabulum. These hooklets possess the usual form met with in the genus.

The conical retractile rostellum is beset with a very great number of hooks, closely arranged in two series. Those belonging to the anterior circlet are larger (·02 mm. long) and alternate with those of the posterior, which are about ·015 mm. in length, measuring from the dorsal root to the ventral root. These hooks are hammer-form, with a long handle or ventral root, and are so closely placed as to form a palisade around the rostellum.

The strobila is narrow (·16 mm. in width), and unsegmented for some little distance behind the scolex. Sexually mature proglottids are relatively short and broad. The genital pores are unilateral, lying in the anterior half of the margin.

Below the thin cuticle is the subcuticula, containing well-defined circular and longitudinal muscle fibres. Calcareous corpuscles are abundant. Lying more deeply there are two well marked series of longitudinal muscles, the outer consisting of numerous smaller bundles forming an almost continuous wide zone, and of an inner group of fewer, but larger, fascicules. Within the latter series lies the well developed transverse musculature.

The main longitudinal nerve is situated some distance dorso-laterally from the wide ventral excretory canal, the latter lying ventrally and laterally from the small dorsal vessel. Between the excretory ducts and above the nerve, there pass outwards the sex canals.

The testes comprise about thirty vesicles (each ·025-·03 mm. by ·015-·02 mm.), arranged in two fields, separated by the female complex, which occupies a central position in the segment. These vesicles are dispersed in the medulla in two or three layers. The vas deferens is recognised as a long convoluted tube lying dorsally, quite close to the anterior border of the segment and extending from the midline, above the ovary, to the cirrus sac. The latter is a small organ, ·10 mm. long by ·04 mm. in width, whose inner extremity does not extend any further than the region of the main nerve. It contains a small vesicula towards its inner end. The sac terminates at the male aperture, situated in front of and slightly ventrally to, the vaginal opening.

As already mentioned, the female complex occupies the middle of the proglottid; the ovary, which lies anteriorly, possesses numerous digitiform lobes directed forwards as well as laterally. Just behind it is the relatively large shell-gland, postero-ventrally to which lies the solid rounded vitellarium. The vagina travels inwards behind the cirrus sac as a wide, more or less straight, tube, which, after passing between the excretory canals, becomes sinuous. Above the ovary it curves backwards to come into relation with the short oviduct and yolk duct, in the vicinity of the shell gland. The uterus, which comes to extend laterally beyond the excretory vessels and the main nerve on each side, consists of numerous egg-capsules, each containing a single egg measuring about ·027 by ·02 mm. The embryo has a diameter of ·017 mm.

Gyrocoelia, sp.

A single specimen, 20 cm. long and 4 mm. in maximum width, was taken from a spur-wing plover, *Lobivanellus lobatus*. The scolex is typical, bearing the zigzag series of hooks on the rostellum. Consideration of this parasite is reserved till a later date.

Bancroftiella glandularis, Fuhrmann

A few cestodes belonging to this species were taken from a blue crane, *Notophoyx novaehollandiae*.

Acanthotaenia (*Ophiotaenia*) *striata*, n. sp.

PLATE VI, FIGS. 2-4

From one of the legless lizards, the Australian slowworm, *Lialis burtonii*. The parasites had been allowed to become more or less dry, and are therefore of little value. The specimens reached a length of 33 mm. or even longer, the breadth being about 1 mm.

The single poorly-preserved scolex examined has a width of 3 mm., the diameter of each of the prominent suckers being ·12 mm. Cuticular ornamentation, such as the presence of spinules, is not recognisable. Segmentation, which succeeds a neck-like constriction, is of the usual Proteocephalid type. Longitudinal striations are present, but these are probably a result of drying. Sexually mature proglottids vary in size and shape, but average about 1·5 mm. in length by 1 mm. in breadth. The genital pores are single and alternate irregularly. They open near the middle of the margin of the segment.

The testes, which occupy two rather narrow lateral fields widely separated along the midline, consist of numerous vesicles, whose average diameter is about ·03 mm. In segments which have reached male maturity the vesicula seminalis seen near the middle of the proglottid, and, overlying the uterus, becomes thrown into a few loose swollen coils, some of which are also present within the cirrus sac. The latter is a pyriform organ ·18 mm. long and

·09 mm. in maximum width, terminating at the male pore postero-dorsally to the female aperture.

The ovary is a transversely-placed bilobed organ, situated close to the posterior end of the segment, and extending across it for a distance of about ·6 mm. Its central portion is very narrow. The vagina travels inwards from the female pore, antero-ventrally to the cirrus sac, then below the vas deferens, curving backwardly to lie above the uterus. It becomes coiled in front of, above, and behind the ovarian bridge. Below the latter the uterus passes forwards, at first as a narrow tube with a sinuous course, but widening suddenly, this broader portion extending as an irregularly pouched median sac almost throughout the length of the segment. The vitellaria occupy a narrow zone on the outer side of each testicular field, the small follicles (·02 mm. diameter) being arranged more or less in a single row dorsally above the nerve. Eggs measure ·03 by ·023 mm., the oncospheres possessing a diameter of about ·013 mm.

Acanthotaenia, sp.

PLATE VI, FIG. 1

A few immature specimens of a Proteocephalid, measuring from 4 mm. long by ·4 mm. wide to 11 mm. by 1 mm., were taken from the intestine of a monitor lizard, *Varanus varius*, Shaw. They are all in the plerocercoid stage, the scolex being distinctly marked off from the remaining unsegmented portion. The posterior end is rounded. The general shape of the scolex is indicated by fig. 1. There is a large apical muscle plug representing a rudimentary sucker.

As the cuticle is practically smooth, it is uncertain whether the parasite should be listed as an *Acanthotaenia* or an *Ophiotaenia*, though the latter is perhaps merely a subgenus of the former.

Acanthotaenia, sp.

From the intestine of a Carpet Snake, *Python variegatus*. This cestode resembles *A. tidswelli* from *Varanus* spp. in its general anatomy and size. As there are several specimens, all of them unaffected by digestive agencies, it is possible that they belong to a species infesting pythons, but I am not aware of any proteocephalids having been described as parasitising this family of snakes. It is therefore possible that these tape-worms may have belonged to a *Varanus* which had formed part of a snake's prey.

Sparganum, sp.

From a frilled lizard, *Chlamydosarus kingii.*

The specimens are fragmentary and twisted after the manner of a corkscrew. The longest piece is about 22 mm. in length and 2 mm. in width. One fragment has a breadth of 3·5 mm.

Tetrarhynchus, sp.

Encysted larval tetrarhynchs were collected from the peritoneal tissues of two fish—a king fish, *Seriola lalandi*, and a bream, *Chrysophrys hasta*. Each larva is enclosed in a thick brownish-yellow elongate bladder or cyst wall. Owing to the scolex being introverted, neither its characters nor those of the long rostella are recognisable, though the whole organ belongs to the *Anthocephalus* type.

ACANTHOCEPHALA

Echinorhynchus, spp.

Hook-headed worms were taken from two birds, the peewhit, *Grallina picata*, and the curlew, *Numenius cyanopus*, but their examination has not been completed.

EXPLANATION OF PLATE VI

REFERENCE TO LETTERING

a.m., apical muscle (rudimentary sucker); c.s., cirrus sac; d.v., dorsal vessel; g.p., genital pore; l.m. 1, l.m. 2, longitudinal muscles; n., nerve; ov., ovary; r., rostellum; r.h., rostellar hooks; s.g., shell gland; t., testis; tr. m., transverse muscle; u., uterus; v., vagina; v.d., vas deferens; vit., vitellarium; v.v., ventral vessel.

Fig. 1. Scolex of immature proteocephalid from *Varanus varius*.

Fig. 2. Segment of *Acanthotaenia* (*Ophiotaenia*) *striata* from *Lialis burtonii*.

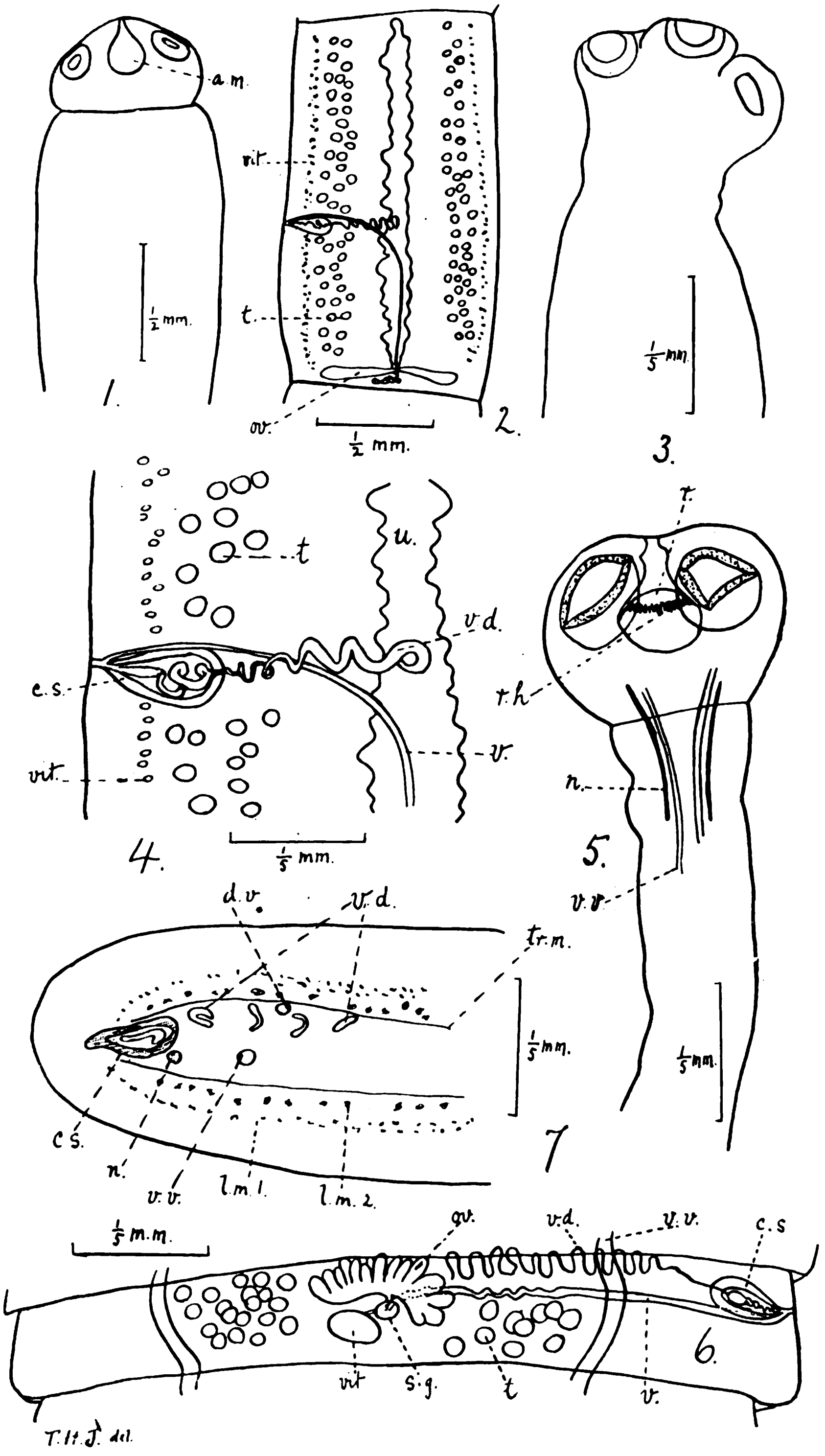
Fig. 3. Scolex of *A. striata*—distorted view, showing three of the suckers.

Fig. 4. Part of segment of *A. striata*.

Fig. 5. Scolex of *Davainea sphecotheridis*.

Fig. 6. Segment of *D. sphecotheridis*.

Fig. 7. Transverse section of portion of segment of *D. sphecotheridis*.



T.H.J. del.

C. Tinling & Co., Ltd., Imp.

EXPLANATION OF PLATE VI

REFERENCE TO LETTERING

a.m., apical muscle (rudimentary sucker); c.s., cirrus sac; d.v., dorsal vessel; g.p., genital pore; l.m. 1, l.m. 2, longitudinal muscles; n., nerve; ov., ovary; r., rostellum; r.h., rostellar hooks; s.g., shell gland; t., testis; tr. m., transverse muscle; u., uterus; v., vagina; v.d., vas deferens; vit., vitellarium; v.v., ventral vessel.

Fig. 1. Scolex of immature proteocephalid from *Varanus varius*.

Fig. 2. Segment of *Acanthotaenia* (*Ophiotaenia*) *striata* from *Lialis burtonii*.

Fig. 3. Scolex of *A. striata*—distorted view, showing three of the suckers.

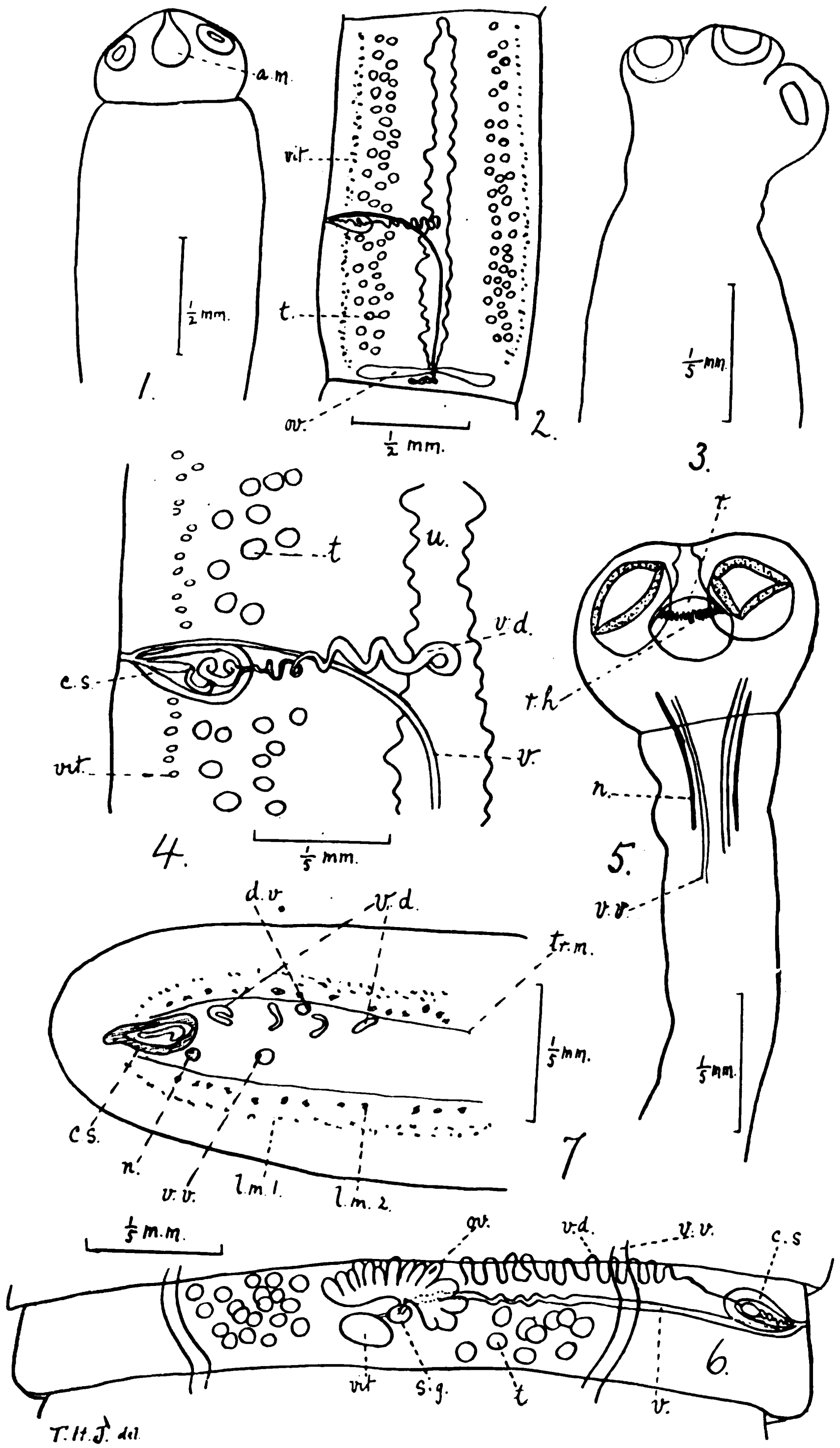
Fig. 4. Part of segment of *A. striata*.

Fig. 5. Scolex of *Davainea sphecotheridis*.

Fig. 6. Segment of *D. sphecotheridis*.

Fig. 7. Transverse section of portion of segment of *D. sphecotheridis*.

PLATE VI



C. Tinling & Co., Ltd., Imp.

MICROSPORON SCORTEUM (N. SP.) FROM A CASE OF RINGWORM IN MAN

BY

HENRY PRIESTLEY, B.Sc., M.B., Ch.M.

(From the Australian Institute of Tropical Medicine, Townsville)

(Received for publication 23 *March,* 1914)

PLATE VII

Skin diseases due to parasitic fungi are not of very frequent occurrence amongst the population of Tropical Queensland. It would, therefore, seem of interest to record a case from whose lesions a fungus new to science has been isolated.

The patient, a boy of about fifteen years of age, showed, at the time of examination, two circular inflamed areas of about 30 mm. in diameter on the skin of the outer side of the right calf. The edges were well defined, and the inflammatory changes were only noticeable in the peripheral parts which were slightly raised, whereas the central portions showed marked pigmentation. There was no scaliness of the affected parts, and the hairs appeared normal to the naked eye.

The patient did not complain of any subjective symptoms beyond slight itchiness of the affected areas.

The lesions cleared up completely within four weeks with no treatment beyond scrubbing with soap and water, leaving no trace beyond a slight pigmentation of the skin.

Another case had occurred previously in the same household, which, unfortunately, did not come under our notice.

Microscopic examination of hairs from the lesions showed them to contain only a very few spores and little mycelium, resembling otherwise a typical microsporon infection.

CULTURAL CHARACTERISTICS

Cultures were made by stabbing hairs from the lesions, well washed with alcohol and ether into the surface of Sabouraud's maltose agar.

The rate of growth of the fungus on *Sabouraud's maltose agar* was remarkable for its rapidity, as already on the second day tubes seeded with hairs showed a distinct growth, sometimes as much as 1 cm. in diameter.

During the first few days the culture was of whitish colour and fluffy, but by about the fifth day it had become a light cinnamon colour, except for the border, which remained white, and then the surface of the growth and its colour gave the impression of the rough side of a piece of chamois leather.

Subcultures on Sabouraud's maltose agar presented the same appearance; on plates the colonies sometimes reached a diameter of 6 cm. in the course of ten days.

Each colony formed a roughly circular disc with uneven edges. The surface, which was without wrinkles, appeared distinctly rough, and showed a small central boss at the site of inoculation, and a fine white duvet at the periphery.

As the cultures aged, the colour deepened and pleomorphism was first noticed at the end of two months, when small tufts of white duvet appeared in different parts of the colonies, which spread until finally the whole surface was covered.

The colonies on *nutrient agar* presented similar appearance; the growth, however, was slower, the roughness of the surface less marked and the colour lighter and sometimes almost white.

In nutrient broth there was a rapid surface growth of a deep cinnamon colour, with numerous folds.

In 1 *per cent. peptone water* the fungus only grew on the surface, the culture presenting the same characters as on Sabouraud's agar. The addition of sugars to the extent of 1 per cent. did not produce any noticeable change in the growth. The sugar, however, disappeared after a time without the production of acid or gas.

Hanging-drop preparations in 1 *per cent. peptone water* were made. In these the multiseptate spores had germinated at the end of six hours, forming mycelial threads, which filled the drop within 18 hours, and at the end of 48 hours a well marked duvet was noticed on the under surface, containing numerous multiseptate spores.

In *milk* rapid growth occurred, the casein being slowly digested without previous clotting.

In a simplified *Raulin's medium* the fungus grew very slowly as a globular, flocculent mass at the bottom of the tube, remarkable for the great number and the size of the chlamydospores it contained. The cultures were grown at room temperature, varying between 20° C. and 30° C.

MORPHOLOGICAL CHARACTERISTICS

The most remarkable feature in the development of this fungus was the early appearance of great numbers of *multiseptate spores*. In hanging-drop preparations they quickly obscured the central part of the culture. A few of these spores are unicellular; the majority have from three to six divisions, and they vary in length from 35 to 60μ, and from 11 to 14μ in width. They may be seen singly or, rarely, in bunches of 6 to 8 at the extremity of filaments. Their ends are smooth, showing no spines.

In most of the cultures *lateral conidia* were scanty. They are, on an average, 3 to 4μ long and 2 to 3μ wide, and grow either as lateral buds from mycelial hyphae or from the free ends of these hyphae. Large grape-like masses have not been observed.

Chlamydospores were infrequent and not sharply defined within the mycelial threads, except in some old cultures.

On one occasion a broth culture of the fungus was kept at 37° C. for several weeks. Microscopic examination of subcultures in hanging drops showed that the mycelial threads consisted of short irregular segments of varying size. In further subcultures the same kind of mycelial threads developed from each segment at first, but after the formation of a number of segments the further growth took on the characters of an ordinary mycelium. The fact that the mycelium grew from these segments shows them to be chlamydospores (Fig. 4).

The presence of abundant *nodular bodies* similar to those described by Sabouraud in *Tr. lacticolor* was an interesting occurrence.

They were very conspicuous, showing most of the forms described by Sabouraud for *Tr. lacticolor*, and, in 16 days' old cultures, in hanging-drop preparations showed an abundant formation of buds and new mycelium (Fig. 5).

Pleomorphism.—The pleomorphic duvet showed a structure which was quite different from the original culture.

The centre of hanging-drop cultures was filled with irregular mycelium, the periphery consisting of radiating mycelial threads, many of them showing clubbed ends.

Spirals were of frequent occurrence in the pleomorphic duvet, although never found in the primary growth. They were very distinct but somewhat irregular and flattened.

Pectinate bodies were only rarely found (Fig. 6).

A certain number of the mycelial threads, both in the primary and in the pleomorphic cultures, showed the formation '*en raquette*,' as described for other species of microsporon.

DISCUSSION

The systematic position of this fungus is difficult to decide, as it presents characters belonging to the trichophytons and characters belonging to the microsporons. The great abundance of the multiseptate spores, the pectinate bodies and the formation 'en raquette' of certain mycelial elements seem to place it among the microsporons. On the other hand, the presence of spirals and nodular bodies and the absence of spines on the multiseptate spores approximate it to the trichophytons.

On the whole, the morphological characters of microsporon are well pronounced, and the occurrence of spirals and nodular bodies alone does not seem evidence enough to consider the fungus a trichophyton. In our opinion, it belongs to the quick growing microsporons of animal origin, but we have been unable to trace the source of infection. Attempts to infect guinea-pigs were unsuccessful.

This microsporon resembles in cultural and morphological characters *M. fulvum*, as described by Uriburu (1909). The description of this fungus, as given by Sabouraud is, however, not sufficiently detailed to decide whether our microsporon is identical with *M. fulvum.*

The presence of spirals which have not been described for *M. fulvum,* and the absence of the fine mycelial outgrowths, such as are seen in *M. fulvum,* seem to justify us in regarding this microsporon as a new species.

On account of the resemblance of the cultures to chamois leather, we propose the name *Microsporon scorteum.*

I am indebted to Dr. Nisbet, of Townsville, for his kindness in drawing my attention to the patient from whose lesions the cultures were obtained.

REFERENCES

SABOURAUD (1910). *Les Teignes.* Paris, 1910.

URIBURU (1909). Argentina Medica. 1909.

EXPLANATION OF PLATE VII

Fig. 1. Ten days' culture of *Microsporon scorteum* on Sabouraud's maltose agar. Natural size.

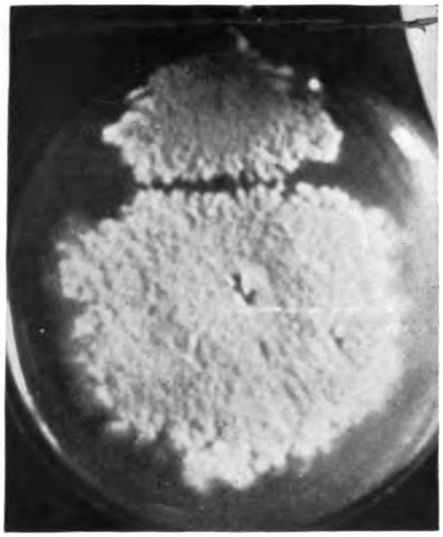
Fig. 2. Ten days' growth in hanging drop 1 per cent. glucose peptone water, showing mycelium and numerous multiseptate spores. Magnification 15.

Fig. 3. The same culture as Figure 2. Magnification 100.

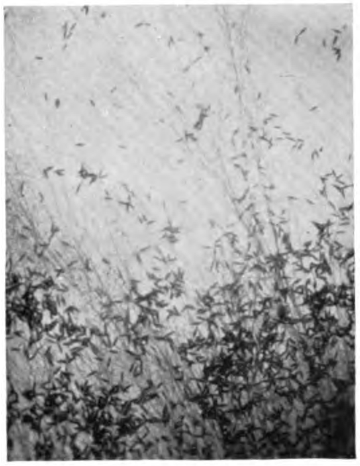
Fig. 4. Chlamydospores (A), showing the development of mycelial threads (B). Magnification 200.

Fig. 5. Nodular bodies. Same culture as Fig. 2. Magnification 100.

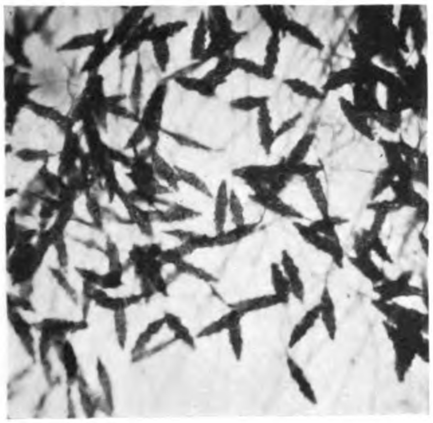
Fig. 6. Pectinate bodies in hanging-drop cultures (1 per cent. glucose peptone water: 4 days' growth). Magnification 100.



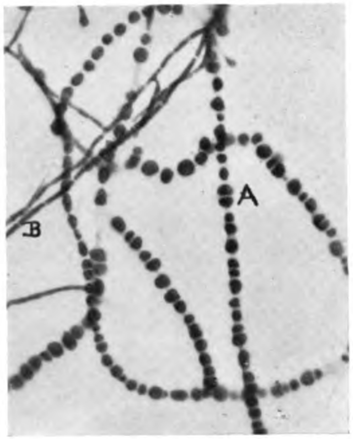
1



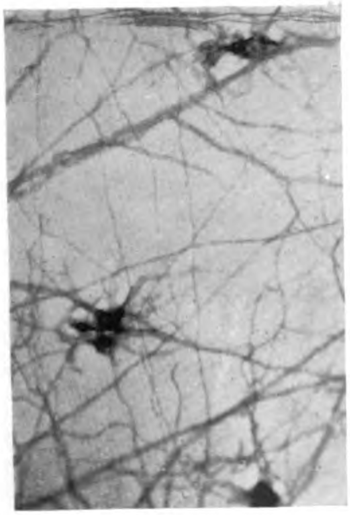
2



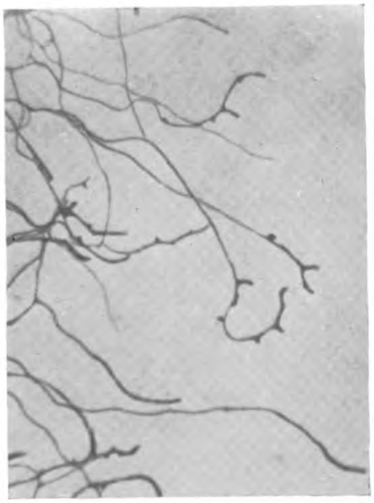
3



4



5



6

C. Tinling & Co., Ltd., Imp.

A NEW MALARIA PARASITE OF MAN*

BY

J. W. W. STEPHENS, M.D.

SIR ALFRED JONES PROFESSOR OF TROPICAL MEDICINE, UNIVERSITY OF LIVERPOOL

(*Received for publication* 19 *January*, 1914)

PLATES VIII-X

In the autumn of 1913 Major Kenrick, I.M.S., kindly sent me, from Pachmari, Central Provinces, India, a blood slide from a native child, containing numerous malaria parasites. On examining these, which I at first took to be malignant tertian parasites, the suspicion arose in my mind that there was something peculiar about their appearance. I happened just previously to have been studying a blood slide from Rhodesia, containing very numerous malignant tertian parasites. The peculiarity of the Indian parasite as far as I could at first define it, was that it was an irregular parasite as compared with the regular, almost monotonous, contour of the 'rings' of the malignant tertian parasite (*Plasmodium falciparum*). I proceeded then to study the Indian parasite more carefully; and, after daily observations for many weeks of it, and of control malignant tertian parasites from various sources, I came definitely to the conclusion that it was unlike any malignant tertian parasite that I had ever seen or that I could find figured in the text-books or journals. I also considered carefully the possibility of its being the simple tertian parasite, but to this point I shall return later. During this study, in order to fix my impressions, I drew 150 consecutive parasites from the Indian slide and the Rhodesian slide respectively, as the former appeared in the field of view of an ocular so restricted by placing a diaphragm in it that only half a dozen red cells were visible in the field at a time, thus effectively preventing any selection on my part. I reproduce as pen-and-ink drawings 35 of each series taken at random as they show very well in a general way the different aspect of the two parasites. For the same purpose I also drew a number of young simple tertian parasites.

---

* Read before the Royal Society on Feb. 19, 1914, and reprinted from *Proc. Roy. Soc.*, B, Vol LXXXVII, pp. 375-377.

I now proceed to define as far as possible in detail the peculiarities of this parasite.

1. It is *extremely amoeboid* (judging from the stained specimens). Thin processes often extend across the cell or occur as long tails to more or less ring-shaped bodies. These processes may be several in number, and may give the parasite most peculiar fantastic shapes like that of an irregular web or mesh.

2. The *cytoplasm* is always scanty, i.e. the individual amoeboid processes are delicate or thin, and the parasite has but little bulk, or density. While forms resembling 'rings' do occur, yet, owing to the abundance of all kinds of irregular forms, it is certainly difficult to find quite typical 'signet' rings. Laterally applied parasites (*accolés* of French authors) also occur, but in them the chromatin is not dot-like, as it usually is in the malignant tertian, but practically always rod-like.

3. The *nuclear chromatin* is out of proportion to the volume of the parasite. It takes the form of bars or rods, strands, curves, forks, patches, etc.; the occurrence of the chromatin in a dot, as in the 'ring' forms of other species, is rare. In the web-like protoplasmic processes mentioned above there may be seen several chromatin strands, and not uncommonly one observes a minute dot of chromatin some way from the parasite, or between two portions of the parasite, though the protoplasmic process connecting it with the main mass or masses is so thin as to be invisible. The chromatin masses are frequently angular, the angles jutting into the points at which an amoeboid process is given off. Abundance of, and marked irregularity in distribution of, the chromatin masses are characteristic of this parasite.

I reproduce in a coloured plate the peculiar forms of this parasite, as it is very difficult, if not impossible, to describe them in words.

I next consider in what respects this parasite, in my opinion, differs from the hitherto described parasites of malaria.

Malignant Tertian Parasite. It differs from this

(1) In its amoeboid activity. In the case of the malignant tertian parasite a certain amount of amoeboid activity is observable,

giving rise to 'star-fish' shapes, and to somewhat irregular or even bacillary forms; but the activity is not comparable with that of this parasite, which has for this reason a most strange and peculiar appearance. The picture produced by the splash of a drop of ink on paper may suggest some of the forms seen.

(2) In the abundance and irregularity of nuclear matter. This, as the coloured plate shows, is very different from what one finds in the malignant tertian parasite, where the term 'signet rings' well expresses the general morphology. The quotidian parasite, if such exists, differs so slightly morphologically from the malignant tertian parasite that the differences just pointed out between this Indian parasite and the malignant tertian apply equally to it.

Simple Tertian Parasite. It differs from this in the following respects: —

(1) Its *bulk* is much less, i.e. it is a smaller parasite.

(2) The *amoeboid processes* are far more delicate.

(3) The *chromatin* shows a relative abundance, an irregularity and a peculiarity of arrangement (e.g. strands, rods and bars) not seen in the simple tertian parasite.

(4) *Typical rings* are absent or exceedingly rare.

I am not sure whether this parasite enlarges the cell, as, although in some cases I found infected cells larger than non-infected ones in their vicinity, in other cases the reverse held good. I am uncertain also whether it is pigmented or not. I have found no parasites in which I could certainly detect pigment; but, on the other hand, I found three pigmented leucocytes in the film, which leucocytes may be associated with this parasite or may result from an associated infection. Finally, I am in doubt as to whether it produces any change in the red cell such as Schüffner's dots. During the course of my examination of this film I must have observed many thousand parasites, but among these I encountered only one infected cell which was clearly enlarged and which showed Schüffner's dots. The bulk of this parasite was much greater than that of any other I had seen, whereas the chromatin masses (two in number, one large, one small) were small compared to the bulk of the parasite. Although I could detect no pigment in this parasite, I was not otherwise able to distinguish it from a simple tertian parasite.* These points then must remain unsettled until further material is forthcoming.

Quartan. Its amoeboid activity and its tenuity easily distinguish it from this species.

After a prolonged study of this parasite I believe then that its morphology differentiates it from any malaria parasite of man yet described.

I propose to call it *Plasmodium tenue*.

---

* I incline to the view, however, that this large form belongs to the other irregular forms. and hence that this parasite enlarges the cell and produces a stippling in it, and hence also that it has affinities with the simple tertian parasite, and *Plasmodium canis* of the dog rather than with the malignant tertian parasite.

DESCRIPTION OF PLATES

The figures were all drawn with an Abbé camera lucida at the same magnification, × 2300 (approx.).

PLATE VIII

Plasmodium falciparum. Blood slide from Rhodesia, 35 parasites drawn at random.

PLATE VIII

Plasmodium falciparum. × 2300. (approx.)

PLATE IX

Plasmodium tenue. Blood slide from Central Provinces, India, 35 parasites drawn at random.

PLATE IX

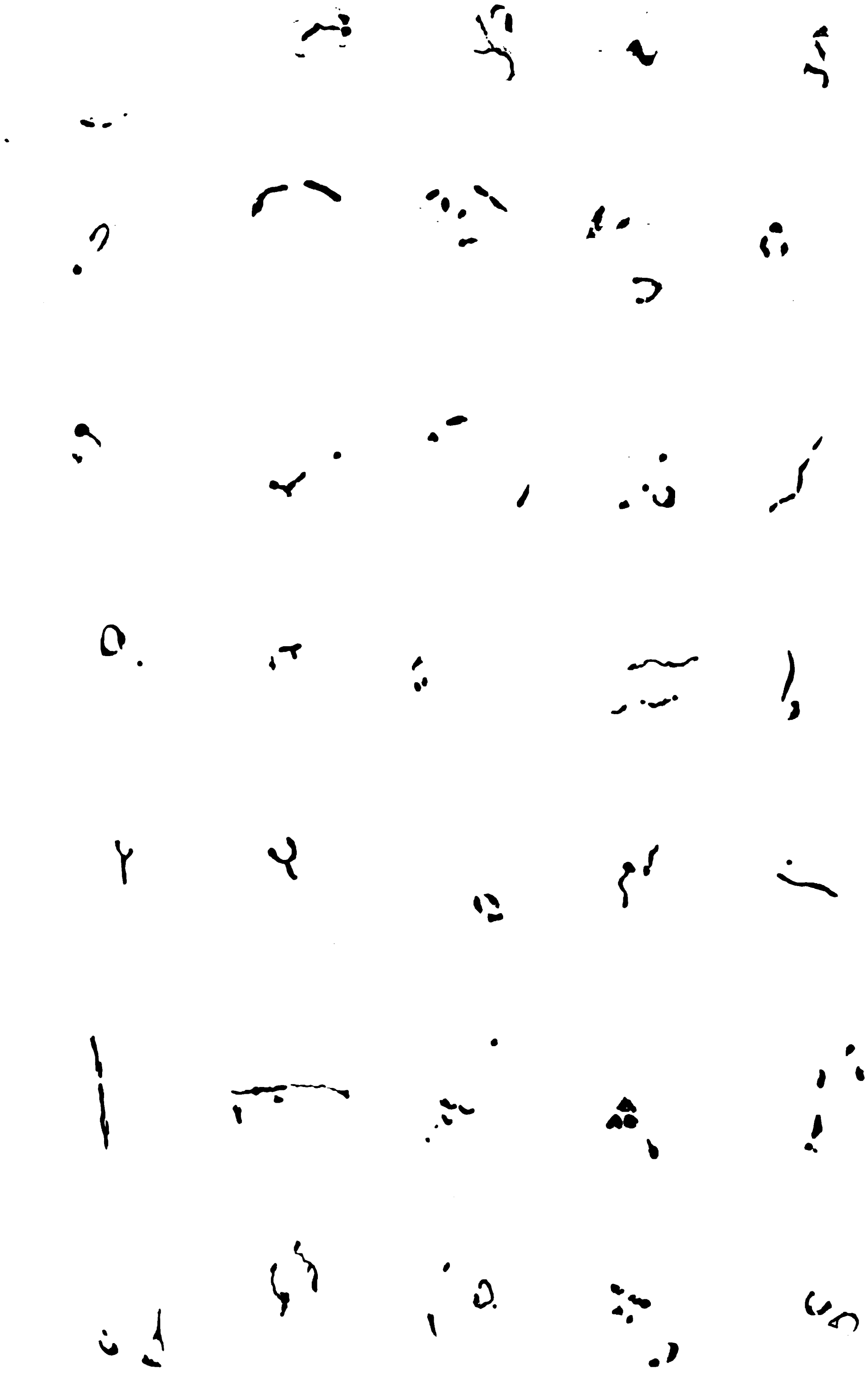
Plasmodium tenue.

x 2300. (approx.)

PLATE X

Plasmodium tenue. Illustrating the irregularity of form of this parasite.

PLATE X



Plasmodium tenue.

X 2300. (approx.)

THE MUTILATION OF WOUNDED ON THE BATTLEFIELD: A CASE DESCRIBED

BY

J. B. CHRISTOPHERSON, M.D. (CANTAB.), F.R.C.P. (LOND.), F.R.C.S. (ENG.)

KHARTOUM, SUDAN

(*Received for publication* 30 *March*, 1914)

PLATE XI

The accompanying photograph (Pl. XI) illustrates the old practice of mutilating the wounded by cutting off the penis and scrotum together with the testicles on the field of battle.

It was taken from an old Sudanese, of about 55 years of age, belonging to the Kongali Tribe of Darfur. He was the eunuch in the household of an Egyptian officer at the time the photograph was taken, and the history of the case was as follows:—

When Ismail Pasha was Governor-General of the Sudan (1873-1877), a battle was fought by the Arabs and Sudanese against King John of Abyssinia at Morab, whereat King John was victorious.

This man, who could not have been more than 17 years of age at the time, was engaged in the fight and received two wounds, one by a sword on the back of the neck, and another on the inner aspect of the right thigh by a bullet.

Whilst lying helpless on the ground he recovered consciousness to find that he had been mutilated. His external organs of generation had been removed.

He was taken prisoner, was with the Abyssinians for 13 years and learnt their language. He afterwards returned to the Sudan.

The sword wound in the neck was dressed with powdered charcoal made from 'Sant' wood, a tree extensively used in the Sudan for medical and commercial purposes.

The other wounds, including the mutilated generative organs, were allowed to take care of themselves, the result being that they were soon crawling with maggots but eventually healed. The ultimate result was as shown in the photograph.

In warfare the Abyssinians used to cut off the generative organs as evidence of having slain an enemy. The trophy was kept and shown on return from battle, and the possessor was rewarded. They mutilated both dead and living wounded whom they could not take prisoners as slaves, such as the Italians at Adua in 1896, but in the case of Sudanese, who are useful as slaves, they spared the living.

The Sudanese from whom the photograph was taken told me that, at the period when he was fighting, the Abyssinian would shoot and, seeing his man fall, he would rush in and, if the man was only wounded, take him prisoner, but if killed he would cut off the penis and testicles as a trophy.

For the photograph the man was seated on a stool in the operating theatre. A piece of indiarubber tubing was inserted into the urethera.

The adductor longus tendons are seen on each side. The raphe of the scrotum is seen passing back to the perinaeum, on each side of the raphe is a roundish fold of scrotum which, however, contained no testicles. The surface marking of Poupart's ligament is seen. The point where the scars converge on the lower part of the abdomen marks about the top of the symphysis pubis. The top of the pubic arch is marked by the piece of indiarubber tubing representing the urethra.

The man consulted me on account of the orifice of the urethra becoming constricted, necessitating the frequent insertion of a piece of old catheter to keep it patent, a practice which resulted in constant attacks of cystitis.

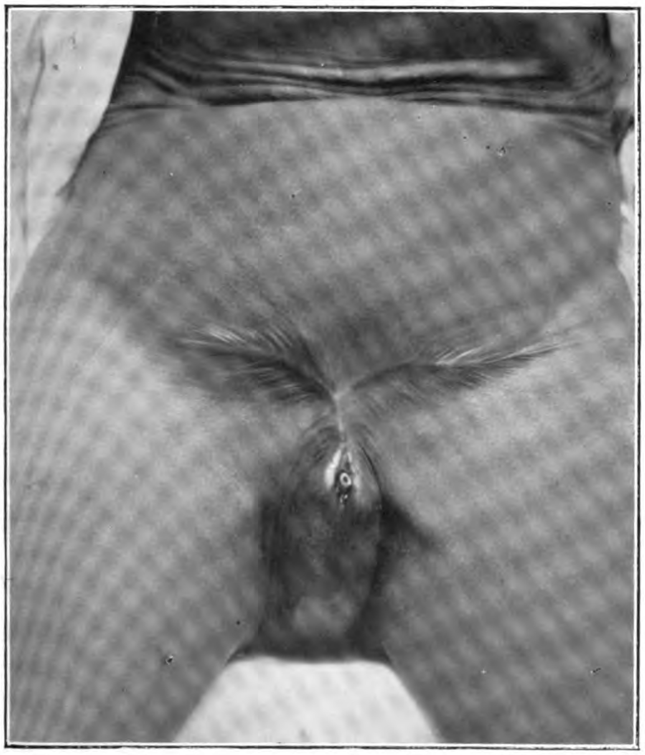
I remedied this by bringing the urethra to the surface in the perinaeum behind the remains of the scrotum, relieving him of the necessity of constantly having to dilate the orifice and of his constant attacks of cystitis, and making the act of micturition a more convenient one.

With regard to his present general condition, it may be said that he was docile, harmless and contented, and was the faithful old servant in the family of an Egyptian officer in the Sudan.

EXPLANATION OF PLATE XI

The photograph illustrates the short account of a Sudanese living at Khartoum, who whilst fighting against the Abyssinians 40 years ago was mutilated by them.

An indiarubber tube is inserted into the orifice of the urethra. There are no testicles in the folds which represent the remains of the scrotum.



C. Tinling & Co., Ltd., Imp

Volume VIII July, 1914 No. 2

ANNALS
OF
TROPICAL MEDICINE AND PARASITOLOGY

ISSUED BY
THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Edited by
PROFESSOR J. W. W. STEPHENS, M.D. Cantab., D.P.H.
PROFESSOR R. NEWSTEAD, M.Sc., J.P., F.R.S., A.L.S., F.E.S., Hon. F.R.H.S.
PROFESSOR WARRINGTON YORKE, M.D.
AND
PROFESSOR SIR RONALD ROSS, K.C.B., F.R.S., M.D., F.R.C.S.,
MAJOR I.M.S. (RET.)

Editorial Secretary
DR. H. B. FANTHAM,
School of Tropical Medicine,
The University,
Liverpool.

C. Tinling & Co., Ltd.
Printers to the University Press of Liverpool
53 Victoria Street

THE INCORPORATED
LIVERPOOL SCHOOL OF TROPICAL MEDICINE

FOUNDED BY SIR ALFRED LEWIS JONES, K.C.M.G.

(Affiliated with the University of Liverpool)

Hon. President: H.R.H. PRINCESS CHRISTIAN OF SCHLESWIG-HOLSTEIN.
Chairman: MR. F. C. DANSON.
Vice-Chairman: Professor R. CATON.
Hon. Vice-Presidents: THE DUKE OF NORTHUMBERLAND, K.G.
THE EARL OF DERBY, G.C.V.O.
EARL CROMER, G.C.B.
SIR EDWARD MEREWETHER, G.C.V.O.
VISCOUNT MILNER, G.C.B.
LORD PIRRIE, K.P.
SIR OWEN PHILIPPS, K.C.M.G.
SIR WILLIAM H. LEVER, BART.
MR. O. HARRISON WILLIAMS

COMMITTEE

Mr. H. J. READ, C.M.G.	*Colonial Office*
Vice-Chancellor SIR ALFRED DALE	*University of Liverpool*
Sir W. B. BOWRING, Bart.	*Council of University of Liverpool*
Professor R. CATON	
Professor W. HERDMAN, F.R.S.	*Senate of University of Liverpool*
Professor J. M. BEATTIE	
Dr. C. J. MACALISTER	*Royal Southern Hospital*
Mr. G. P. NEWBOLT	
Mr. C. BOOTH (Jun.)	*Steamship Owners' Association*
Mr. T. F. HARRISON	
Mr. A. R. MARSHALL	*Shipowners' Association*
Mr. W. ROBERTS	
Mr. J. W. ALSOP	
Mr. G. BROCKLEHURST	
Mr. C. LIVINGSTON	
Mr. J. W. W. DANSON	
Professor E. W. HOPE	
Mr. R. RANKIN	
Professor E. GLYNN	
Mr. E. G. BUCKLEY	
Mr. A. L. REA, *Hon. Treasurer*	
Mr. A. H. MILNE, C.M.G., *Secretary.*	

B 10 Exchange Buildings, Liverpool

Staff, 1914

1. At the University of Liverpool

Professors - -	JOHN WILLIAM WATSON STEPHENS, M.D., Cantab., D.P.H. *Sir Alfred Jones Professor of Tropical Medicine* ROBERT NEWSTEAD, F.R.S., M.Sc., A.L.S., F.E.S., *Dutton Memorial Professor of Entomology* MAJOR SIR RONALD ROSS, K.C.B., F.R.S., LL.D., F.R.C.S., D.P.H., M.D., D.Sc. Nobel Laureate 1902. (Indian Medical Service, retired). *Professor of Tropical Sanitation* WARRINGTON YORKE, M.D., *Walter Myers Professor in Parasitology*
Lecturers - -	HAROLD BENJAMIN FANTHAM, D.Sc., B.A., *Lecturer in Parasitology* HENRY FRANCIS CARTER, S.E.A.C. DIPL., F.E.S., *Lecturer in Entomology* WILLIAM THOMAS PROUT, M.B., C.M.G., *Lecturer on Tropical Sanitation* HAROLD WOLFERSTAN THOMAS, M.D., C.M. PROF. E. W. HOPE, M.D., D.Sc., *Lecturer on Municipal Sanitation*
Honorary Lecturer -	MAJOR JOSEPH FITZGERALD BLOOD, M.D., M.Ch. (Indian Medical Service, retired)
Honorary Statistician	WALTER STOTT
Bibliographer - -	WALTER DRAWZ

2. At the Yellow Fever Bureau

HARALD SEIDELIN, M.D. (Scientific Secretary).

3. At the Runcorn Research Laboratories

Director - - -	B. BLACKLOCK, M.D., D.P.H.
Assistant - - -	Vacant.

4. At the Royal Southern Hospital, Liverpool

Physicians - -	CHARLES JOHN MACALISTER, M.D., F.R.C.P. JOHN LLOYD ROBERTS, M.D., M.R.C.P.
Surgeons - - -	D. DOUGLAS CRAWFORD, F.R.C.S. ROBERT JONES, F.R.C.S., Ch.M. GEORGE PALMERSTON NEWBOLT, M.B., F.R.C.S.
Tropical Pathologist -	JOHN WILLIAM WATSON STEPHENS, M.D. Cantab.

5. On Expedition

HAROLD WOLFERSTAN THOMAS, M.D. C.M.

Laboratory
JOHNSTON LABORATORY, UNIVERSITY OF LIVERPOOL

Research Laboratory:
RUNCORN

Hospital:
ROYAL SOUTHERN HOSPITAL, LIVERPOOL

Secretary's Office:
B 10, EXCHANGE BUILDINGS, LIVERPOOL

NOTICE

The following courses of instruction will be given by the Liverpool School of Tropical Medicine during 1914:—

Full Course begins 6 January. Advanced Course begins 1 June.
Diploma Examination, 6 April. Certificate Examination, 30 June.
Full Course begins 15 September.
Diploma Examination, 14 December.
These dates are subject to revision.

The full Course of Instruction is open to all qualified medical men, and the Examination to all students who have taken out this full course.

Fee for the full Course of Instruction—Thirteen Guineas.

Fee for the Diploma Examination—Five Guineas.

Fee for the Short Course of Instruction—Four Guineas.

Fee for the use of a School microscope during one term—Ten shillings and sixpence.

For prospectus and further information, application should be made to the Dean of the Medical Faculty, University of Liverpool.

The following have obtained the Diploma in Tropical Medicine of the University of Liverpool:—

Diploma in Tropical Medicine

Date of Diploma	
1904	Augustine, Henry Joshua
1904	Bennett, Arthur King
1904	Bruce, William James
1904	Byrne, John Scott
1904	Clayton, Thomas Morrison
1904	Dalziel, John McEwen
1904	Dee, Peter
1904	Greenidge, Oliver Campbell
1904	Hehir, Patrick
1904	Khan, Saiduzzafor
1904	Laurie, Robert
1904	Maclurkin, Alfred Robert
1904	McConnell, Robert Ernest
1904	Nicholson, James Edward
1904	Philipson, Nicholas
1904	Sharman, Eric Harding
1904	Thomson, Frank Wyville
1904	Walker, George Francis Clegg
1905	Anderson, Catherine Elmslie
1905	Brown, Alexander
1905	Caldwell, Thomas Cathcart
1905	Critien, Attilio
1905	Hooton, Alfred
1905	Hudson, Charles Tilson
1905	Illington, Edmund Moritz
1905	Macfarlane, Robert Maxwell
1905	Maddock, Edward Cecil Gordon
1905	Moore, James Jackson
1905	Nightingale, Samuel Shore
1905	Radcliffe, Percy Alexander Hurst
1905	Young, John Cameron
1906	Adie, Joseph Rosamond
1906	Arnold, Frank Arthur
1906	Bate, John Brabant
1906	Bennetts, Harold Graves
1906	Carter, Robert Markham
1906	Chisholm, James Alexander
1906	Clements, Robert William
1906	Dundas, James
1906	Faichnie, Norman
1906	Jeffreys, Herbert Castelman
1906	Mackenzie, Donald Francis
1906	Pailthorpe, Mary Elizabeth
1906	Palmer, Harold Thornbury
1906	Pearse, Albert
1906	Sampey, Alexander William
1906	Smithson, Arthur Ernest
1906	Taylor, Joseph van Someron
1906	Taylor, William Irwin
1906	Tynan, Edward Joseph

Date of Diploma	
1906	Watson, Cecil Francis
1906	Willcocks, Roger Durant
1906	Williamson, George Alexander
1907	Allan, Alexander Smith
1907	Allwood, James Aldred
1907	Bond, Ashton
1907	Branch, Stanley
1907	Collinson, Walter Julius
1907	Davey, John Bernard
1907	Donaldson, Anson Scott
1907	Fell, Matthew Henry Gregson
1907	Gann, Thomas William Francis
1907	Graham, James Drummond
1907	Hiscock, Robert Carroll
1907	Keane, Joseph Gerald
1907	Kennan, Richard Henry
1907	Kenrick, William Hamilton
1907	Le Fanu, George Ernest Hugh
1907	Mackey, Charles
1907	Maddox, Ralph Henry
1907	McCarthy, John McDonald
1907	Raikes, Cuthbert Taunton
1907	Ryan, Joseph Charles
1907	Vallance, Hugh
1908	Caverhill, Austin Mack
1908	Crawford, Gilbert Stewart
1908	Dalal, Kaikhusroo Rustomji
1908	Dansey-Browning, George
1908	Davidson, James
1908	Dickson, John Rhodes
1908	Dowdall, Arthur Melville
1908	Glover, Henry Joseph
1908	Greaves, Francis Wood
1908	Goodbody, Cecil Maurice
1908	Harrison, James Herbert Hugh
1908	Joshi, Lemuel Lucas
1908	Le Fanu, Cecil Vivian
1908	Luethgen, Carl Wilhelm Ludwig
1908	Mama, Jamshed Byramji
1908	McCay, Frederick William
1908	McLellan, Samuel Wilson
1908	Pearce, Charles Ross
1908	Schoorel, Alexander Frederik
1908	Smith, John Macgregor
1908	Stewart, George Edward
1908	Tate, Gerald William
1908	Whyte, Robert
1909	Abercrombie, Rudolph George
1909	Allin, John Richard Percy
1909	Armstrong, Edward Randolph
1909	Barrow, Harold Percy Waller
1909	Beatty, Guy
1909	Carr-White, Percy
1909	Chevallier, Claude Lionel
1909	Clark, William Scott
1909	Cope, Ricardo
1909	Fleming, William
1909	Hanschell, Hother McCormick
1909	Hayward, William Davey
1909	Henry, Sydney Alexander
1909	Innes, Francis Alexander
1909	Jackson, Arthur Frame
1909	Kaka, Sorabji Manekji
1909	McCabe-Dallas, Alfred Alexander Donald
1909	Meldrum, William Percy
1909	Murphy, John Cullinan
1909	Samuel, Mysore Gnanandaraju
1909	Shroff, Kawasjee Byramjee
1909	Thornely, Michael Harris
1909	Turkhud, Violet Ackroyd
1909	Webb, William Spinks
1909	Yen, Fu-Chun
1910	Brabazon, Edward
1910	Castellino, Louis
1910	Caulcrick, James Akilade
1910	Dowden, Richard
1910	Haigh, William Edwin
1910	Hamilton, Henry Fleming
1910	Hefferman, William St. Michael
1910	Hipwell, Abraham
1910	Homer, Jonathan
1910	Houston, William Mitchell
1910	James, William Robert Wallace
1910	Johnstone, David Patrick
1910	Korke, Vishnu Tatyaji
1910	Macdonald, Angus Graham
1910	Macfie, John Wm. Scott
1910	Manuk, Mack Walter
1910	Murison, Cecil Charles
1910	Nanavati, Kishavlal Balabhai
1910	Nauss, Ralph Welty
1910	Oakley, Philip Douglas
1910	Pratt, Ishmael Charles
1910	Sabastian, Thiruchelvam
1910	Shaw, Hugh Thomas
1910	Sieger, Edward Louis
1910	Sousa, Pascal John de
1910	Souza, Antonio Bernardo de
1910	Waterhouse, John Howard
1910	White, Maurice Forbes
1911	Blacklock, Breadalbane
1911	Brown, Frederick Forrest
1911	Chand, Diwan Jai
1911	Holmes, John Morgan
1911	Ievers, Charles Langley
1911	Iles, Charles Cochrane
1911	Ingram, Alexander
1911	Kirkwood, Thomas
1911	Knowles, Benjamin
1911	Liddle, George Marcus Berkeley
1911	Lomas, Emanuel Kenworthy
1911	Mackarell, William Wright
1911	MacKnight, Dundas Simpson
1911	Mascarenhas, Joseph Victor
1911	Murray, Ronald Roderick
1911	Oluwole, Akidiya Ladapo
1911	Rao, Koka Ahobala
1911	Sinton, John Alexander
1911	Tarapurvalla, Byramji Shavakshah
1911	Taylor, John Archibald
1911	Woods, William Medlicott

Date of Diploma	
1912	Aeria, Joseph Reginald
1912	Anderson, Edmund Litchfield
1912	Borle, James
1912	Bowie, John Tait
1912	Brassey, Laurence Percival
1912	Christie, David
1912	Dillon, Henry de Courcy
1912	Dunn, Lillie Eleanor
1912	Hardwicke, Charles
1912	Jagose, Jamshed Rustomji
1912	Kochhar, Mela Ram
1912	McGusty, Victor William Tighe
1912	Milne, Arthur James
1912	Mitra, Manmatha Nath
1912	Myles, Charles Duncan
1912	Pelly, Huntly Nevins
1912	Prasad, Bindeshwari
1912	Prentice, George
1912	Ross, Frank
1912	Russell, Alexander James Hutchison
1912	Ruthven, Morton Wood
1912	Sandilands, John
1912	Seddon, Harold
1912	Smalley, James
1912	Strickland, Percy Charles Hutchison
1912	Watson, William Russel
1913	Austin, Charles Miller
1913	Banker, Shiavux Sorabji
1913	Becker, Johann Gerhardus
1913	Carrasco, Milton
1913	Clark, James McKillican
1913	Forsyth, Charles
1913	Grahame, Malcolm Claude Russell
1913	Grieve, Kelburne King
1913	Hargreaves, Alfred Ridley
1913	Hepper, Evelyn Charles
1913	Hiranand, Pandit
1913	Jackson, Oswald Egbert
1913	Khaw, Ignatius Oo Kek
1913	MacKelvie, Maxwell
1913	MacKinnon, John MacPhail
1913	Macmillan, Robert James Alan
1913	Mouat-Biggs, Charles Edward Forbes
1913	Noronha, John Carmel
1913	O'Connor, Edward
1913	Olubomi-Beckley, Emanuel
1913	Pestonji, Ardeshir Behramshah
1913	Puttanna, Dodballapur Sivappa
1913	Reford, John Hope
1913	Smith, Edward Arthur
1913	Stewart, Samuel Dudley
1913	Walker, Frederick Dearden
1913	Wilbe, Ernest Edward
1913	Wilson, Hubert Francis
1913	Yin, Ulg Ba
1913	Young, William Alexander
1914	Arculli, Hassan el
1914	Chohan, Noormahomed Kasembha
1914	Connell, Harry Bertram
1914	Gwynne, Joseph Robert
1914	Hodkinson, Samuel Paterson
1914	Jackson, Arthur Ivan
1914	Kaushash, Ram Chander
1914	Kelsall, Charles
1914	Luanco y Cuenca, Maximino
1914	Rowe, John Joseph Stephen
1914	Sur, Sachindra Nath
1914	Wilkinson, Arthur Geden

EDITORIAL NOTICE

By order of the Committee of the Incorporated Liverpool School of Tropical Medicine, the series of the Reports of the School, which had been issued since 1899, were followed, from January 1, 1907, by the Annals of Tropical Medicine and Parasitology, of which this is the second number of the eighth volume.

Altogether twenty-one Memoirs, besides other works, were published by the School since 1899, and of these ten, containing 519 quarto or octavo pages and 95 plates and figures, were published during the two years 1904 and 1905.

The Annals are issued by the Committee of the School, and will contain all such matter as was formerly printed in the Reports—that is to say, accounts of the various expeditions of the School and of the scientific work done in its laboratories at the University of Liverpool and at Runcorn. In addition, however, to School work, original articles from outside on any subject connected with Tropical Medicine and Hygiene, and Parasitology may be published if found suitable (see notice on back of cover); so that, in all probability, not less than four numbers of the Annals will be issued annually. Each number will be brought out when material sufficient for it has been accumulated.

With reference to the article in the last number of the *Annals of Tropical Medicine and Parasitology*, dated 21st April, 1914, entitled 'Observations on a so-called cure for Trypanosomiasis' by Warrington Yorke and B. Blacklock our attention has been called by Dr. Mehnarto to a construction which has been placed upon the article which we did not intend to convey.

The serum experimented with was not supplied by Dr. Mehnarto and the article in question was not intended in any way to imply that this particular serum was prepared by him.

The serum mentioned in the article was supplied by Mr. Sieg alone and we regret if the article has caused Dr. Mehnarto any annoyance.

A STUDY OF THE *ENTAMOEBAE* OF MAN IN THE PANAMA CANAL ZONE

BY

WILLIAM M. JAMES, M.D. (VIRGINIA)

ANCON HOSPITAL, ANCON, CANAL ZONE, PANAMA.

(*Received for publication* 6 *January*, 1914)

PLATES XII-XVII

CONTENTS

PAGE

PREFACE ... 134

PART I—A SUMMARY OF THE MORE IMPORTANT LITERATURE ... 136
- MORPHOLOGY AND DEVELOPMENT OF *Entamoeba tetragena* ... 151
  - Vegetative Forms ... 151
  - Degeneration Forms ... 154
  - Chromidia and Cyst Formation ... 156

PART II—MORPHOLOGY OF THE ENTAMOEBAE FOUND IN THE CANAL ZONE ... 163
- Introduction ... 163
- Material ... 166
- Morphology ... 167
- Entamoebae associated with Mild, or with Latent Dysentery ... 180
- *Entamoeba coli*... 200
  - Distribution ... 203
  - Size ... 203
  - Cytoplasm and Motion ... 206
  - Nucleus ... 213
  - Multiplication ... 222
  - Free Nuclei and Enucleate Amoebae ... 228
  - Cyst Formation ... 231
  - Encystment ... 239
- Entamoebae resembling *E. coli*, but classified as Separate Species ... 251

PART III—CLASSIFICATION OF THE ENTAMOEBAE OF MAN FOUND IN THE CANAL ZONE 253
- GROUP I ... 254
  - Entamoebae associated with Mild, or Latent Dysentery ... 265
  - Classification of Group I—Entamoebae whose end stage in the Human Host is a four-nucleated cyst ... 266
  - Relation of Dysenteries to the types of Entamoebae encountered... 269
  - Correlation of the Types of Entamoebae ... 270
  - The so-called Chromidia Formation in the Entamoebae ... 275

PART IV—THE TECHNIQUE OF VARIOUS METHODS USED IN FIXING AND STAINING THE ENTAMOEBAE ... 282
- Wet Fixation and Staining Methods ... 283
- Staining Methods ... 290

BIBLIOGRAPHY ... 305

EXPLANATION OF PLATES ... 310

PREFACE

It is my purpose in this contribution to place before those interested in amoebic or tropical dysentery, from the point of view of the practising physician rather than from that of the protozoologist, such facts as are of interest in the identification and classification of the entamoebae of man.

Recent work in the study of these organisms has resulted in the acceptance by most authorities of a unity of the pathogenic species, with the name of *Entamoeba histolytica*. As is known, however, the pathogenic entamoebae vary greatly in virulence, morphology, and in the phases of their cycles as these are found in man. The variations in morphology and in the phases of the cycles have in the past brought about a diversity of species, under the names of *E. histolytica*, *E. tetragena*, *E. minuta*, and others; and, although the non-pathogenic *E. coli* is less subject to variation than the pathogenic entamoebae, some writers have included under the head of non-pathogenic entamoebae several species.

Nearly all the types and species described by others have been found in the Panama Canal Zone. Some of these are unquestionably pathogenic, while others are certainly harmless. But however much importance may be given to the correct classification of species, the differentiation between the various types of pathogenic entamoebae and those of the non-pathogenic entamoebae has a practical value of equal importance, whether there be one or several species of each, and this differentiation I have endeavoured to obtain by a study of the entamoebae as I have found them in health and in disease.

Although as a result of my study I am of the opinion that there are two species only of entamoebae found in man—a pathogenic species, *E. histolytica*, whose cycle ends in a four-nucleated cyst, and a non-pathogenic species, *E. coli*, whose cycle ends in an eight-nucleated cyst—I have found it convenient in writing of the types of entamoebae encountered to call these by the names with which they are commonly known at the present time. So that, when writing of *E. histolytica*, *E. tetragena* and *E. minuta*, I wish to be understood as describing what appear to me as different types of the same species. So well defined are these three types, and so intimately are they related to various clinical aspects of amoebic dysentery, that I believe it is justifiable to classify them as distinct types, and to write of them as such, since to describe all of the protean forms of the pathogenic species under one name would at the present time confuse those who have not followed closely the work done in the past two years.

At the same time, I wish to state that the classification of species is not as yet fully determined, and is a task for experts in protozoology, rather than for those like myself, who, however many different kinds of entamoebae they may have seen, have not had the opportunity to devote themselves to a study which requires so much time and so great an experience. My reasons for believing in one species only of pathogenic entamoeba are set forth in Part III of this contribution, but I think it is of far more importance to the practising physician to recognize a pathogenic entamoeba than to concern himself with the unity or plurality of species. For this reason I have, as stated above, endeavoured to describe the entamoebae and to illustrate them in a manner that will, I hope, be of assistance to those who wish to know at the earliest moment possible whether or not they have to do with a pathogenic infection.

I have followed Prof. E. A. Minchin's use of the adjective 'chromatinic' instead of 'chromatic,' which latter until recently has been in common use.

I wish here to acknowledge the kindness of Dr. W. E. Deeks, Chief of Medical Clinic in Ancon Hospital, at whose request I took up this study. It is due to him that I could devote so much of my time for the past two years to it, and in recognition of his unfailing interest and sympathy it is my privilege to dedicate this contribution to him.

I am indebted also to Col. W. C. Gorgas, Chief Sanitary Officer, Isthmian Canal Commission, and to Lieut.-Col. Charles F. Mason, Superintendent of Ancon Hospital, for their permission to devote time to this work; also to Col. John L. Phillips, Acting Chief Sanitary Officer, for permission to publish this paper. All three of the above gentlemen are of the Medical Corps, United States Army.

PART I

A SUMMARY OF THE MORE IMPORTANT LITERATURE

Authorities who are conversant with the growth of our knowledge of the *Entamoebae* are unanimous in assigning to the well-known work of Schaudinn (1903) the first certain differentiation of these organisms, as they occur in the intestine of man, into a pathogenic and a non-pathogenic species. Jürgens (1902) had given an excellent account of the pathogenic species, while Casagrandi and Barbagallo (1897) had described and figured various phases in the life cycle of what is now known as *E. coli.*

Schaudinn, however, was the first to separate the two species by a study of the life cycle of each, as well as by certain morphological characteristics. The pathogenic species he named *Entamoeba histolytica.* This organism he characterised by an eccentrically placed, chromatin-poor nucleus, easily distorted by internal pressure, and not readily observed in fresh preparations. The nuclear membrane is single in optical section, and very delicate. The ectoplasm, even in the resting state, is well differentiated from the endoplasm; the former being homogeneous, highly refractive, and plainly set off from the latter, which is less refractive and granular. This entamoeba reproduces by simple division, by budding and by an autogamous (?) spore formation, which latter is preceded by a distribution of the chromatin from the nucleus to the cytoplasm. These clumps of chromatin make their way to the periphery of the organism, and there are enfolded in the ectoplasm, the resulting spores being thrown off by a process of budding.

The non-pathogenic species, to which Schaudinn gave the name of *Entamoeba coli*, he described as having also well defined morphological and developmental characteristics. The nucleus is rich in chromatin, and is readily seen in fresh preparations. In optical section, a stout and well defined double contoured membrane is made out, and the nucleus does not lose its rounded shape from internal pressure. The organism moves slowly, first in one direction, and then in another, and does not exhibit progressive motion. The ectoplasm is less refractive than the endoplasm, and is seen only during movement. Large vacuoles, which enclose food débris, are frequently observed. Reproduction takes place by simple division; by schizogony, with formation of eight daughter entamoebae; and by cyst formation, in which there is autogamous union of the nuclei after reduction.* The end stage is the formation of an eight-nucleated cyst, which is characteristic of the species.

Schaudinn also proved the pathogenicity of *histolytica*, and the non-pathogenicity of *coli*, by a series of interesting experiments, to which I shall refer later.

Although more recent work tends to discredit several of Schaudinn's conceptions as to the ontology of the two species, particularly his description of reproduction in *histolytica*, his morphological observations are in all essentials correct, and to-day can be used as criteria in distinguishing between *coli* and the one or more pathogenic species.

Schaudinn's important work, to which high praise has been given by subsequent students of the entamoebae, does not appear to have attracted much attention at the time. To judge by the recent literature lists, from 1903 to 1907 only a few workers sought to confirm his findings.

In 1905 Craig, who had an exceptional opportunity to study the entamoebae, published the results of his observations in nearly 1,500 cases of amoebic dysentery, which he had studied since 1901, not only in endemic centres of the disease in the Philippines, but in San Francisco also, where many patients suffering from chronic cases were invalided. These observations I hold to be of very great importance, since Craig could study so many cases both of acute and chronic amoebic dysentery, an opportunity not to be had in Europe or elsewhere in the United States, as a study of the recent literature will show.

Craig fully confirmed the existence of two species, one pathogenic and the other non-pathogenic, and observed also the morphological characteristics and reproductive phases of these, as described by Schaudinn. Craig also called attention to the rapid progressive motion of *histolytica*, and to the greenish tint often seen in this organism when blood is present in the stools, as aids in identification. He was the first, to my knowledge, to study and describe the entamoebae after dry fixation and staining by one of the modifications of the Romanowski method. Although many recent writers have sought to discredit the results obtained by this method of study, it is, as I shall endeavour to demonstrate in Part IV, a simple and valuable aid in distinguishing *E. coli* from the pathogenic species, whatever may be its value for critical cytological study.

---

* Autogamy is denied by Hartmann, see pp. 236-239.

The classification of the *Entamoebae* as given by Schaudinn appears to have been accepted, until Viereck (1907) published his communication. Not only is Viereck's work of highest importance in that he first recognised the four-nucleated cysts now held to be characteristic of the species called *tetragena*, but he also described variations in the morphology of the vegetative pathogenic organisms from the type described by Schaudinn as *histolytica*. This latter fact has an important significance, as will be shown later, and seems to have been somewhat overlooked by those who take the cycle of *E. tetragena*, as described by Hartmann (1912), for the type of the pathogenic species.

Viereck notes that visibility or invisibility of the nucleus in fresh preparations depends on certain properties of the organism, such as the abundance of endoplasm, the amount of contained food, and on the density of the endoplasm itself. Although it had been commonly said that the nucleus of a pathogenic entamoeba is difficult to see, in his opinion it can be easily made out with a good microscope. It lies eccentrically, and contains blocks of chromatin, often on the circumference, which are visible on account of their high refraction. The organisms undergoing reproduction have complicated nuclei, whose study is difficult, but necessary.

Viereck states that Schaudinn is not correct in assuming only one pathogenic entamoeba, *E. histolytica*, and only one not pathogenic, parasitic entamoeba, *E. coli*. Viereck observed a variety of *E. coli* which appeared to have pathogenic properties. In this type the reduction and regenerative processes took place in the undivided nuclei (I think this means without autogamy), and the fully developed cysts had only four nuclei. Eight-nucleated cysts are characteristic of *coli*, while permanent forms of *histolytica* are produced by budding. Viereck here notes a point of importance—that in order to see this budding, it is necessary to observe it in fresh preparations, and then to fix and stain these for further study.

He states that the nucleus of *histolytica*, as described by Schaudinn, is poor in chromatin, inconstant in position, and has an ill-defined membrane. On the other hand, the nucleus of the vegetative form of *coli* is rich in chromatin, central or sub-central in the organism, and has a well marked membrane.

In two of his cases Viereck observed entamoebae 'which are to be assigned to those described by Casagrandi and Barbagallo (*Entamoeba coli*, Schaudinn),' because the protoplasm was but little refractive and the nucleus rich in chromatin, so that a well-defined network was made out, and cysts were formed. These entamoebae were variable in size, but in general the diameter was somewhat longer than usually observed. The ectoplasm was less refractive than the endoplasm, and could be made out only when in motion. Specimens wet fixed in sublimate-alcohol and stained with hematoxylin presented a honeycomb structure of the cytoplasm. In the vegetative stage the nucleus had a net-like formation, with granules of chromatin scattered over the network. Forms were also seen with the chromatin in large bars around the nuclear periphery. Compact, dumb-bell shaped nuclei were likewise observed, suggesting mitosis. Cyst formation, however, was not the same as in *E. coli*, for the cysts never contained more than four nuclei.

Viereck could not find any evidence of autogamy in the cyst formation. According to his account, the cysts are at first so full of chromidia that it is difficult to make out the nucleus, but as the chromidia condense, the nucleus becomes more easily observed. He states that the 'Innenkörper' (probably the karyosome) plays an important part in the further process of cyst formation. It increases in size, and a large nucleus is formed, which is dotted evenly with chromatin granules. In a later stage these granules arrange themselves in a spiral thread, which divides into four shorter threads. These four threads form rings, which at first are associated in pairs, but later separate. Viereck believes that the entamoeba just described was seen also by Casagrandi and Barbagallo.

He observed also, in other cases of amoebic dysentery, organisms that by reason of their nuclear appearance were clearly not *histolytica*, and since they did not form cysts could not be classed as *E. coli*. He found these organisms in chronic, relapsing cases, in which no dysentery bacilli could be demonstrated in the stools, and whose serum did not agglutinate the strains of dysentery bacilli at his disposal. Although the nuclei of these organisms had a very marked resemblance to those of *E. coli*, Viereck doubts whether the organisms should be classed with this species. He states that Kruse and Pasquale (1893-94) had described similar organisms, and that Craig had also seen them, and associated them with dysentery. Quinke and Roos (1893), on the other hand, called the pathogenic organisms observed by them *Amoebae coli*, Loesch.

Viereck then notes that the entamoebae described by other authors may be separated as follows. *E. histolytica* (pathogenic) and *E. coli* (harmless) of Schaudinn, and *E. coli* (pathogenic) of Kruse and Pasquale and of Quinke and Roos. Apparently, from this last group other species may be separated. By their pathogenicity they are differentiated from the harmless *E. coli*, and by the abundance of nuclear chromatin from the vegetative forms of *E. histolytica*.

In the pathogenic entamoebae described by Jürgens (1902), Viereck states, the ectoplasm and the endoplasm are always clearly differentiated; in those described by Kruse and Pasquale only when the organisms are in motion. Jürgens found the endoplasm always granular and free of vacuoles; Kruse and Pasquale found it either structureless or granular and vacuolated, as did Casagrandi and Barbagallo. Jürgens described the ectoplasm as more refractive than the endoplasm; Kruse and Pasquale found the contrary. The amoeboid movement, with which the flowing of the protoplasm begins, takes place, according to Jürgens, in the endoplasm; according to Kruse and Pasquale, in the ectoplasm. Jürgens stated that the nucleus, when stained, shows only the membrane and the 'Kernkörperchen' (chromatinic granules, and probably a small karyosome); Kruse and Pasquale, that it is frequently granular. In Viereck's cases a honeycomb nuclear structure was found.

By reason, however, of the four-nucleated cysts, which hitherto had not been described for the entamoebae of man, Viereck believed that he had found a new species, and named it *Entamoeba tetragena*.

Viereck's observations lose none of their importance in the light of more recent findings. He was the first to note that the vegetative stages of certain entamoebae associated with dysentery differed from Schaudinn's description, and he called attention to the variations also described by earlier authors.

In 1907, also, Hartmann, with v. Prowazek, found an entamoeba in some cases invalided home from Africa, which, by reason of its nuclear structure, he could differentiate from the harmless *coli* and the pathogenic *histolytica*. He considered this a new species, and named it *E. africana*, but shortly after (1908), finding four-nucleated cysts, he was convinced that he had to do with the same organism that Viereck had studied, and withdrew his name in favour of Viereck's *tetragena*.

In 1908 Craig published an account of his further researches. In this he noted certain variations from type in both *histolytica* and *coli*, so that the examination of a few organisms could not be held as a sure guide to differentiation.

In this year Werner (1908) confirmed the existence of *histolytica* as described by Schaudinn, and of *tetragena* as described by Viereck and by Hartmann. Werner's morphological studies on *tetragena* confirmed in all essential details Hartmann's account of the vegetative stage of *tetragena*. He states that especially characteristic for this species is the cycle of the karyosome. He does not describe cyst formation, and states that he had not observed this. He pictures, however, a four-nucleated vegetative organism that closely resembles some stages in cyst formation.

Werner states that the identification of *histolytica*, like that of the vegetative stage of *tetragena*, is made on the morphological occurrences in the nucleus. The lack of nuclear membrane; the chromatin, scanty in comparison with that of *tetragena*, heaped in blocks on the nuclear membrane; and the little karyosome, with the perceptible inconstancy of form and ill-defined nucleus during life, give supporting points for a differentiation of species. Werner's contribution is well illustrated, and his pictures have definite value in determining the significance of 'reproduction by budding.'

In 1909 several papers of interest appeared, the most important being those by Hartmann, Elmassian and Noc.

Hartmann's (1909) paper on *histolytica* is the first of his proposed series dealing with the entamoebae parasitic in man. He notes that while Schaudinn was the first clearly to demonstrate a differentiation of species, he by no means settled definitely the question of how many species there may be. In the first place, Schaudinn's paper was a preliminary communication (vorlaüfige Mitteilung) without illustrations, and many workers, only superficially understanding his descriptions of structure and of multiplication processes, drew erroneous conclusions, thus helping to confuse what knowledge really was available. In the second place, since Schaudinn's publication another pathogenic entamoeba, *E. tetragena*, has been described. Hartmann states that Schaudinn later knew of this other species.

It is very likely, I believe, that had Schaudinn lived, the dispute that exists to-day between those who not only hold that *histolytica* is a species, but also that one of its methods of reproduction is the spore formation described by him, and those who claim that *tetragena* is the only pathogenic species, would have been decided some time ago. The impartial critic should bear in mind Hartmann's statement, which is also confirmed by Werner (1908), that the celebrated paper which has lately caused so much controversy was a preliminary communication. Indeed, in all fairness, it may be said that one of Schaudinn's most important experiments has never been confirmed, as will be shown later.

Hartmann says that Schaudinn's work has been confirmed only by Craig (1908), Werner (1908), and himself (1907-1908). Here the author overlooks Craig's important communication in 1905, which was the first confirmation in English of Schaudinn's findings, and certainly one of the first in any language. Hartmann states that Craig's confirmation of Schaudinn's reproductive phases of *histolytica* is inaccurate, by reason of the dry fixation technique employed. He does not agree that the substance which Craig considered as the chromidia really is chromidia. He also states that most of Werner's pictures of chromidial formation in *histolytica* in reality represent degenerating forms; and he adds that the accounts of Kartulis, Ruge, and Noc do not permit of a differentiation between *histolytica* and *tetragena*.

Hartmann's own preparations, upon which he based his study of *histolytica*, consisted of fresh material from one case only, which had originated in China; and stained specimens, the greater part of which had been left by Schaudinn. The exact number of cases is not given. His description of what he considered as *histolytica* is as follows.

Size. E. histolytica is the smallest of the three hitherto described entamoebae parasitic in the intestine of man. The organism enlarges as the stage of chromidial formation is reached, in contradistinction to *tetragena*, which is smaller at this stage. The appearance of the entamoebae in life has been correctly described by Jürgens and by Schaudinn, so that there is but little to add. The ectoplasm is differentiated from the endoplasm even in the resting stage. While this differentiation offers an aid in distinguishing *histolytica* from *coli*, the study of living organisms only may at times lead to confusion; for, by reason of changes in the surrounding medium and in the internal economy of the entamoebae themselves, the relation between ectoplasm and endoplasm may be altered, as Hartmann has frequently observed in *tetragena*.

The Nucleus. The nucleus of the vegetative *histolytica*, Hartmann states, is extraordinarily characteristic, as was noted by Schaudinn. Contrary to that of *tetragena*, and of *coli*, the nucleus of *histolytica*, in fresh preparations, does not have a double contoured achromatinic membrane, which is observed frequently in the other species. For this reason the nucleus is sometimes distorted when in motion by the foreign bodies in the endoplasm, as can be observed sometimes during life. Its position is always eccentric, often at the margin of the ectoplasm, against which it not infrequently appears pressed into a disc shape. This appearance is not seen in the other species.

In stained preparations these alterations in shape are equally well observed. While in *tetragena* and *coli* the nucleus always appears round or oval, in *histolytica* such a shape is seldom found, and often the nucleus is greatly distorted. Further, its lack of chromatin is striking and characteristic; there is at the most, as Schaudinn has described, only a little karyosome, and, on the margin of the nucleus, a delicate, condensed layer of chromatinic substance, which latter may be mistaken for a nuclear membrane.

As a rule the little karyosome has but scanty chromatin, and the centriole, which is so often seen in *tetragena*, is but seldom found. The cyclical changes of the karyosome, which according to Hartmann and v. Prowazek, are characteristic of all true karyosomes, are very infrequently found in *histolytica;* while in *tetragena* these changes are very marked, and, as noted, often serve for a direct method of differentiation. In *histolytica* only a delicate ring of chromatin between the karyosome and the outer nucleus can be made out at times.

Hartmann states that he has never observed in the living organism either the budding or the nuclear division described by Schaudinn; nor has any such confirmation been made by others. He regards the observations of Lesage (1905) and Noc (1909) as having to do with degeneration forms, or with cells other than entamoebae. However, Craig (1905) described in detail simple division in the living organism, but states that budding was not observed in fresh preparations.

Hartmann says that he has seen the phenomenon described by Schaudinn as chromidia and cyst formation in two cases. The process begins in a few individuals, and a few days later has appeared in all. It is initiated by the approach of the karyosome to the nuclear membrane, and the giving out of chromatin to the endoplasm. Perhaps the so-called chromidial granules originate exclusively through karyosome division; so that it is not a question of true chromidial budding, but of one or several extrusions of little nuclei, each of which has a potential power of further development.

The remainder of Hartmann's paper concerns the fate of the chromidia, and the so-called bud formation. He is very conservative in his opinion as to this process, but appears to believe that the extrusion of the nuclear contents into the endoplasm is primarily responsible for the subsequent occurrences. In this part of his paper one detects a reluctance to attribute to chromidial formation any importance with regard to propagation of the species by budding.

Hartmann also gives pictures of a possible conjugation: in his fig. 24 of two organisms, and in his fig. 25 of four. He adds that, like Schaudinn, he could not follow the fate of the 'cysts' formed by budding. He did not have the opportunity to confirm Schaudinn's experiments by infecting kittens *per os* with these 'cysts,' on account of lack of material. He states further, 'Schaudinn's account of this point is so precise, that there is no room for doubt as to the correctness of the experiments.'

Noc's (1909) account is a very interesting contribution from the viewpoint of the epidemiology of amoebic dysentery and the possibility of culturing the entamoebae associated with this disease. Unfortunately, many of his illustrations, as Hartmann notes, do not permit of a clear distinction between free living amoebae, entamoebae and tissue cells. His paper, however, is of importance in that, although he had abundant material, he could not confirm Schaudinn's statement that *histolytica* forms cysts by budding. Noc notes that this budding does not take place in freshly passed stools, but only after the preparations have been observed for two to three hours, and he quotes Schaudinn as follows:—

'Little by little,' says Schaudinn, 'in two to three hours, by reason of the influence of active currents in the endoplasm, several little buds are thrown out. These arrive at the periphery, and finally are expelled as spheres, from 3 to 7 μ in diameter.' Noc did not consider these buds as true cysts, but gives an account of the formation of little entamoebae from the parent organism without any stage of cyst formation.

I have given these data, because in my opinion, as will be seen later, they are of greatest importance with respect to a proper determination of the so-called reproduction in *histolytica* by 'bud' or 'spore' formation.

In 1909 Elmassian described a new species, which he called *Entamoeba minuta*. Notwithstanding the very evident careful research which was the foundation of Elmassian's observations, his account was not received with the attention due to so exact a presentation. In the past year an entamoeba has been found in the Canal Zone that answers very closely to his description of *Ent. minuta*, and as a full description of *minuta* and an account of Elmassian's opinions will be given in Part II, I shall not go into further detail at present, except to say that from the point of view of previous classifications, *E. minuta* is entitled to recognition equally with the other species.

The contribution of Walker* (1911) is, I believe, one of the most important additions to the literature of the *Entamoebae*. He was the first to show, as will be pointed out, the necessity of regarding ***histolytica*** and ***tetragena*** as different phases of the same organism. He also demonstrated, by a very careful series of experiments, as Craig had previously done, the lack of any close relation between free living and pathogenic amoebae.

He found living amoebae in fresh stools from 142 persons without symptoms of dysentery; from 28 persons with amoebic dysentery; and from the pus in two cases of liver abscess. Fixed and stained specimens were made from 21 cases of amoebiasis without symptoms of dysentery; from 16 cases of amoebic dysentery, and from the pus of the two cases of liver abscess. Sections from the abscess walls and ulcers of the gut were also studied.

Walker's technique in wet fixation differs somewhat from that commonly used, and is described in Part IV.

One type of organism was found exclusively in all the material examined from human sources. It differs from the *Amoeba* type found in the water supply and cultivated from the intestinal tract of man in certain fundamental morphological and biological particulars. The nucleus of the trophozoite is situated eccentrically in the resting organism; the nuclear chromatin is arranged peripherally instead of centrally, as in the culture amoebae; there is no contractile vacuole; reproduction by sporulation does not occur; and multiplication does not take place on ordinary artificial media. The cysts contain four or eight nuclei instead of a single nucleus; a reproductive process takes place within the cyst; and the process of encystment always begins in the host. The author believes that these characteristics are sufficiently constant to justify the establishment of the genus *Entamoeba*. He did not find organisms of the *Amoeba* (free living) type in any of his preparations from stools or liver abscess pus, and he believes that *Amoebae* cultivated from these sources by himself and others are at most only temporary commensals; or, as Werner has suggested, arise from cysts ingested with water or food, passing unchanged through the intestinal tract.

In the stools from the non-dysenteric cases the entamoebae

---

* It is to be regretted that Walker and Sellards' (1913) important paper on 'Experimental Entamoebic Dysentery,' *Philip. Jl. Sc.*, B, VII, p. 253, was received too late for notice in this memoir.

correspond in part with *E. coli*, Schaudinn, and in part with *E. nipponica*, Koidzumi. Two varieties of distribution of nuclear chromatin are distinguished. In the first, the chromatin in optical section appears as continuous, segmented, or granular ring of approximately uniform thickness on the inner surface of the nuclear membrane, with or without some granular chromatin scattered in the nucleoplasmic network or collected in a small karyosome. This variety corresponds to the *coli* species of Schaudinn. In the second variety the chromatin is collected in several discrete clumps on the nuclear membrane, corresponding to the *nipponica* species of Koidzumi.

Walker observed only simple division in the trophozoite, and did not see any evidence of an eight-nucleated schizogony. The mature cysts of each variety contain eight nuclei, sometimes more. Entamoebae of the *coli* and *nipponica* varieties were found only in the stools of healthy persons, or those suffering from diseases other than dysentery. Double infections with the other type of entamoeba about to be described were not found.

The entamoebae found in the stools from cases of amoebic dysentery; in the pus of liver abscesses; and in sections from amoebic lesions in the gut present certain morphological differences from those found in non-dysenteric stools. These differences appear to be fairly constant, and in their sum serve to distinguish the entamoebae from the two sources. The characteristics of the organisms from the dysenteric material correspond in part to those of *E. histolytica*, Schaudinn, and in part to those of *E. tetragena*, Viereck. The author's description of the living organisms in amoebic dysentery is practically identical with that of Schaudinn. He makes no mention of finding a nucleus in fresh preparations corresponding to that described by Hartmann for *E. tetragena*, except as above noted.

In wet fixed and stained preparations Walker found in the dysentery entamoebae also two varieties of distribution of nuclear chromatin. In one the chromatin is extremely scanty, and is arranged as a barely perceptible layer under the nuclear membrane, with or without a small karyosome. This variety corresponds to that described by Schaudinn for the nucleus of *histolytica*. In the other the chromatin is more abundant, and is arranged in part as a granular layer under the nuclear membrane, and in part as a loose central karyosome, which, in its most typical form, consists of a minute centriole surrounded by an achromatinic halo that is bounded by a circle of chromatinic granules. This variety corresponds to that described by Viereck and Hartmann for *tetragena*.

When the acute symptoms have passed, and the stools are becoming normal, the trophozoites are smaller, and the chromatin more abundant. These changes are preparatory to the development of cysts. Such forms, Walker states, resemble *minuta* as described by Elmassian. Walker's staining solution, aqueous alum-hematoxylin, did not demonstrate chromidia at any time in the cycle, so that he does not believe these chromidia are in any way derived from nuclear chromatin.

In the end stage, the cysts contain four nuclei. These nuclei have more chromatin than those of the eight-nucleated cysts found in non-dysenteric stools. In two cases relapses were observed. In one, while the stools contained four-nucleated cysts, symptoms of dysentery appeared, and entamoebae of the *histolytica* type were found. The symptoms abated without treatment, the stools became normal, and four-nucleated cysts were again found. In the second case, during an acute attack the *histolytica* type was observed. Under treatment the stools became normal, and four-nucleated cysts appeared. Later there was a relapse, with the appearance of entamoebae of the *histolytica* type. When the stools were normal again, the four-nucleated cysts re-appeared.

Entamoebae of the *histolytica* and *tetragena* types were found only in stools or tissues of persons who were suffering from an attack of amoebic dysentery, or who had a history of such an attack. The *histolytica* type, Walker found the more common in Manila, which is contrary to Whitmore's (1911) observations. The *tetragena* variety, when found, has been associated invariably with organisms of the *histolytica* type, and transitional forms were common. Also, as the author shows, the *tetragena* cysts were found during relapse associated with *histolytica* vegetative entamoebae.

In view of these observations, Walker is of the opinion that there are only two species of entamoebae parasitic in man. One of these is non-pathogenic, the *Entamoeba coli* of Schaudinn. The other includes the *histolytica* variety of Schaudinn, and the *tetragena* variety of Viereck and Hartmann, and probably the *minuta* variety of Elmassian. He does not think the differential diagnosis between pathogenic and non-pathogenic species an easy matter.

Whitmore (1911) had collected in Manila and Saigon a supply of material from dysenteric and non-dysenteric cases. This included material from 14 cases of amoebic dysentery. He took this material to Berlin, where he studied it in Hartmann's laboratory. His conclusions, confirmed by Hartmann, are that only *tetragena* forms were found in his specimens. The identification was made, when cysts were not present, by the nuclear structure. In one case it was thought that only *histolytica*-type organisms were present, but a prolonged search demonstrated several typical *tetragena* cysts.

In 1912 Hartmann published the results of his more recent studies. This paper, by reason of the authority of its author and its careful attention to detail, has attracted much attention from those who are interested in the study and classification of the entamoebae of man. Not only are several older conceptions, once of much importance, set aside, but the opinion is expressed, although not definitely stated, that there is but one species of entamoeba pathogenic for man. I am certain that much of the future work on this subject, at least for some time, will have to do with confirmation or refutation of Hartmann's views, and for that reason I give here as complete an abstract as possible of the more important statements in his paper, especially as I shall have occasion throughout my own work constantly to refer to them. Credit, however, should not be taken from the careful research of Walker, who, although he did not go into the study of the cycle as fully as does Hartmann, yet based his opinion of the identity of *tetragena* and *histolytica* on quite as careful a research, and on much more material.

Repeated studies of his own and Schaudinn's preparations, Hartmann begins, convinced him that, with one exception, the organisms hitherto described by both of them as *histolytica* were in reality degenerating forms of *tetragena*. This conviction the author could confirm after a study of Whitmore's preparations.

This study showed that in all the cases of amoebic dysentery investigated, *tetragena* was the organism present. Also, by a study of Whitmore's preparations and by further work on his own, he is now of the opinion that autogamy, as previously described by him for *tetragena* cysts, does not happen.

From his study of the preparations left by Schaudinn, Hartmann believes that the latter interpreted vegetative forms of *tetragena* sometimes for *histolytica*, sometimes for *coli*. From this he argues that Schaudinn did not have *histolytica* at his disposal, but *tetragena*.

The case described by Jürgens (1907) is also one of *tetragena*, not of *histolytica*, because later Hartmann had the opportunity to observe this patient for a considerable time. From this case he infected cats, and witnessed the beginning of cyst formation. The amoebae described by Doflein (1909) as *histolytica* are also *tetragena*, since he had the opportunity to study the material on which Doflein based his observations. The author's study of the accounts of Noc (1909) and Ruge (1906) convinced him that these authors also had to do with *tetragena*. His opinion, from these observations, is best expressed in his own words:—

'I can, therefore, at present assert with great surety, on the grounds of a very rich material for study from different tropical countries . . . that almost all cases of amoebic dysentery are not brought about by *E. histolytica*, but by *E. tetragena*.'

The organism described by Koidzumi (1909) as a new species Hartmann considers to represent degeneration in *coli* or *tetragena*, or else degeneration in amoeba-like body cells. The *E. minuta* of Elmassian, he states, is identical with *coli*.

Material.

1. Cases from the 'Militarlazaret,' on which he based his preliminary researches. These cases were of importance, because he could get material almost daily from one of them, and so follow it for two months.
2. Jürgen's case, on which this author based his second publication.
3. Two cases in Rio Janeiro.
4. An autochthonous case, infected in Germany.
5. Fixed and stained preparations; especially those of Schaudinn and Viereck.

MORPHOLOGY AND DEVELOPMENT OF *E. TETRAGENA*

1. *Vegetative Forms*

Size. In fresh preparations, when only vegetative forms are found, the size is about 25 to 40 μ. Prior to cyst formation, the organisms become smaller, and are usually under 20μ. The smallest individuals observed were 5 to 10μ. Size, therefore, is not of importance in the identification of species.

Cytoplasm and Motility. The appearance of *tetragena* agrees entirely with the descriptions that Jürgens (1902) and Schaudinn (1903) have so carefully given for *E. histolytica.* Since these authors, on the whole, clearly had to do with *tetragena*, and not *histolytica*, this is not singular. As Schaudinn knew, the principal distinction between *tetragena* and the harmless *coli* is that in the former there is a plain distinction between ectoplasm and endoplasm in the resting stage. The ectoplasm is highly refractive, while the endoplasm is but little refractive, and contains granules, vacuoles, and food remains. Although in normal individuals the difference between ectoplasm and endoplasm is constant, after some time the organisms lose this characteristic, and many can be found in which there is no differentiation. This happens also in artificial infections in cats. The study of the entamoebae in cats shows many degenerative forms, and it is in these that the differentiation is frequently lost. For this reason the differentiation between ectoplasm and endoplasm is not always a sure guide to identification; one must study also the nuclear structure, propagation, etc.

As a rule only erythrocytes are used as food. In contradistinction to *coli*, bacteria and the remains of these are but seldom found in the endoplasm. Sometimes the entamoebae are stuffed with erythrocytes in various phases of digestion. Large vacuoles containing aggregations of bacteria, such as are often found in *coli*, are not seen in *tetragena.*

The movement is by pseudopodia formed first of the ectoplasm, into which the endoplasm flows. Several varieties of pseudopodia formation are described by the author.

Nucleus. Hartmann describes in great detail the nuclear structure, and the phenomena observed during what he calls the 'cycle of the karyosome.' I shall present here only the more important points.

In case the entamoebae do not contain very many erythrocytes or other food particles in the endoplasm, during life the nucleus is easily seen. It appears as a stout, rounded vesicle, which is plainly marked off from the endoplasm by a well-defined, double-contoured membrane. In the living organism the finer structures of the nucleus can plainly be made out. In by far the greater number of individuals there is, in the centre, a little karyosome, surrounded by a clear, structureless zone. Between this zone and the nuclear membrane is a honeycomb-like structure of linin, on which are placed granules of chromatin.

In the living organism Hartmann could observe some phases of the cycle of the karyosome. The chromatinic substance is at first collected on the outer border of the karyosome, and flows to the periphery as little drops or threads.

This nuclear structure can be taken as characteristic for normal vegetative organisms of 20 to 40 μ. Occasionally smaller forms are found, especially in cat infections, which show a nucleus that is nearly all karyosome (Caryosomekern), without any chromatin in the outer nucleus (Aussenkern). This structure Hartmann describes for the beginning of the karyosome cycle, when all of the chromatin is arranged as a large karyosome, in the centre of which a granule can be seen, the centriole. That part of the nucleus between the outer border of the karyosome and the nuclear membrane—the Aussenkern, as Hartmann calls it—is made up of the honeycomb structure (Wabenreihe).*

The chromatin of the karyosome now breaks up into granules, especially at the outer border, and these, passing outward, appear as little granulations on the nodes of the network, and on the inner margin of the nuclear membrane. At the same time the centriole increases in size, to form a new karyosome; so that when the chromatin of the original karyosome is all distributed to the outer nucleus, a new karyosome is being formed, while of the original karyosome only the outer margin remains, as a delicate chromatinic ring. Thus there arises finally, by the centrifugal, cyclical conversion of the original karyosome, and by the centripetal increase in the centriole to form a new karyosome, the typical nucleus of the adult entamoeba.*

---

* This word, I think, refers to the achromatinic linin network.—W. M. J.

In this, for the most part, the karyosome contains a central granule, the centriole. The typical nucleus of the adult vegetative organism, then, consists of a centriole, with a clear zone around it, surrounded by the karyosome, which latter is limited by the outer border of the original karyosome. This border often simulates a true membrane. In the outer nucleus the chromatin is arranged in dots and threads on the achromatinic network; in dots and granules on the inner surface of the nuclear membrane; or in large plates or bars on this surface. Variations from this type occur, since this arrangement of the nuclear contents is not permanent. The cycle continually repeats itself, so that sometimes three concentric karyosome borders are seen. All of these changes are very beautifully shown in Hartmann's figs. 7-16.

According to Hartmann, these cyclical changes are morphologically extraordinarily well defined, more so than in any other entamoeba. It is true that they are seen in other species, but not so well as in *tetragena*. By reason of these cyclical occurrences the *tetragena* can be differentiated from *histolytica* and *coli*, and especially can a differentiation be made between pathogenic and non-pathogenic entamoebae, as was shown by experiments in Hartmann's laboratory.

Propagation. Hartmann has observed only binary, or simple division in the vegetative forms. His opinion is that division takes place very rapidly in these, since in the examination of many specimens only a few stages were found. His observations show that division begins by a splitting of the centriole. The two centrioles then proceed to the poles of the karyosome, and for a time are connected by a thread which is called the 'centrodesmose.' The karyosome then lengthens, and the entire nucleus is elongated. A part of this process was followed in the living organism. In none of the specimens, however, were the later stages of division seen, nor were several of the intermediate.

---

* I use here Hartmann's phraseology, although in English the adjective 'centripetal' would not be applied, in my judgment, to the increase in size of the centriole, since this increase is apparently from within outward.—W. M. J.

Collection of the chromatin in large blocks on the nuclear membrane, such as was described by Schaudinn for the stage preceding schizogony in *E. coli*, was frequently observed. In Hartmann's opinion, however, this has nothing whatever to do with a multiple nuclear division; but is either a phase of the karyosome cycle, or else a mark of degeneration.

2. *Degeneration Forms*

In most cases of amoebic dysentery, the structure described above as typical of ***tetragena*** is found in the greater part of the entamoebae in the stools. Yet not seldom individuals are met with, more or less frequently, with anomalous cytoplasmic and nuclear appearances. These appearances, as they pertain to movement and to the cytoplasm, have been described. In the entamoebae in which they occur, the nucleus as a rule is not altered, so that by attention to its structure a correct diagnosis can be made.

But in the inner organs, in the intestinal ulcers and in the liver, also in the stools of artificially infected cats, and in human stools prior to encystment of the entamoebae, there are many organisms with anomalous nuclear appearances. Such organisms have caused errors in differentiation, and it is necessary, therefore, Hartmann says, to describe them minutely.

With respect to entamoebae found in the intestines of artificially infected cats, the author notes that they should not be taken for normal individuals. The entamoebae do not find a normal medium in the intestines of cats, and so degenerate. All cytological and developmental studies on entamoebae from cats are to be taken with the greatest reserve. Such organisms very often show the nuclear characteristics that Schaudinn has described for ***histolytica***. It appears certain to the author, that all the entamoebae described by Werner as *histolytica* are degenerating forms. Similar nuclear pictures are found in sections through intestinal ulcers and liver abscesses. Hartmann states that he himself took such pictures as characteristic for *histolytica* in his former publication (1909).

Since in all amoebic dysentery cases of like origin, in which simultaneous observations could be made on preparations from the faeces, the typical *tetragena* characteristics were found, it seems probable that so-called *histolytica* characteristics in these cases are to be attributed only to the fact that the entamoebae showing them were inhabitants of the tissues rather than of the lumen of the canal, and had escaped from their normal environment. However, the author's researches on this point are not yet completed.

Except in cat infections, Hartmann found degenerating forms mostly in human stools shortly before encystment. These forms are as a rule smaller than normal. Among the many degenerating entamoebae thus found, two principal types may be differentiated. In one of these the nucleus is greatly distended, and the chromatin is collected in the form of single large blocks under the nuclear membrane, while the karyosome disappears almost entirely. Such forms are similar to those described by Koidzumi (1909) for his *E. nipponica.*

In the second type there is an inversion of the outer nucleus and of the nuclear membrane as well; these disappear entirely, or almost entirely, so that only a very large karyosome remains.* Although seldom, transitional forms between this and the normal nucleus are found. Such forms, with apparently almost compact nuclei, Koidzumi has described for the true vegetative organisms of *nipponica*, which later go on to schizogony. In so far as it is a question of dysenteric entamoebae, and not body cells, he has been deceived by degeneration forms.

Very often two similar, apparently compact, nuclei in close apposition are found. Formerly such an occurrence caused Hartmann to believe that he had to do with autogamous nuclear copulation. Although since then appearances have been noted which might be interpreted as a reduction division, Hartmann now holds an entirely different opinion. The similarity of these forms to the manifold degeneration stages is evident, and the same is true for all stages which might be interpreted as reduction division.

It would have been easy, Hartmann states, to construct from his preparations an apparently uniform series of pictures of progressive nuclear reduction and division, and dissolution of chromatin. Such a representation, however, would not have been accurate. For the same reason all the accounts and pictures of reduction and division in the cysts of the entamoebae, especially those of Elmassian for his so-called *E. minuta*, which indeed is identical with *E. coli*, are to be viewed critically.

---

* Nuclei of this type resemble closely those of the free living species.—W. M. J.

3. *Chromidia and Cyst Formation*

Cyst formation, according to Hartmann, is an occurrence that happens very seldom in *tetragena* infections. He himself never observed it, although he followed cases daily for weeks and months He observed the beginning of the process once, in a cat infection. For this reason his studies are based on fixed preparations. In all he had at his disposal five cases of cyst formation; one from Egypt, one from Manila, and the other three from Viereck's cases. With the exception of these cases, Hartmann finds cyst formation reported only once, by Huber (1909).

As noted, cyst formation is preceded by a diminution in the size of the entamoebae, and at the same time the chromidia make their appearance in the cytoplasm. All the organisms are affected at the same time, so that after a few days the normal vegetative individuals are no longer seen in the stools. Coincidently the number of the entamoebae increases, for which reason one may assume that the organisms come out from the ulcers and enter upon encystment. If this be true, then encystment is *en rapport* with natural healing. Hartmann does not assert this, and states that further observations are necessary.

The characteristic chromidia are formed at first from the nuclei of the little entamoebae that are about to encyst. Shortly before or during encystment the chromidia collect into compact bars and ovoids. The purpose of this chromidia formation is not entirely clear, but very likely the chromidia play a part in the nutrition of the cyst, since they are used up by the time the end stage is attained. In the chromidia forming organisms the once plain nucleus becomes indistinct, and the nucleus may lose its rounded shape, and appear distorted. Such nuclear structure shows a full agreement with that described by Schaudinn for *histolytica*, as Hartmann could state from his examination of the former's slides. Schaudinn, says Hartmann, took the normal vegetative organisms in these specimens for *E. coli*, as he was working only with fixed preparations in this stage. This clears up Schaudinn's statement that he found a double infection with *coli* and *histolytica*, something never seen by Hartmann. In one of Whitmore's series of specimens, Hartmann also took such organisms for *histolytica*, until, after a prolonged search, *tetragena* cysts were found.

Nuclear division in the cysts is similar to that in the vegetative organisms. Here also is found an intra-nuclear spindle with centrioles at the ends, and threads of chromatin arranged in long rows from pole to pole. A differentiation from vegetative nuclear division is sometimes observed, when in the cysts the spindle is extraordinarily long drawn out.

The nucleus then divides and the daughter nuclei go into a resting stage, in which the karyosome, in comparison with the centriole, is not very plain, since it is concealed by a central collection of chromatin arranged in single granules, for the most part of the same size. Each of the two nuclei then divides, and the end stage, the four-nucleated cyst, is formed.

By reason of its four nuclei in the end stage, the important large chromidial bodies in the earlier phases of cyst formation and its smaller size, the cyst of *E. tetragena* is fairly well differentiated from that of *E. coli.* The large blocks of chromidia are for the most part absent from the latter.

It is Hartmann's opinion that these cysts burst after ingestion in the intestinal canal of a freshly infected person, and little gametes are formed, which copulate one with another, as described by Mercier (1910) for *Entamoeba blattae.*

Wenyon (1912) brings forward several interesting points. He considers that it is highly probable that Schaudinn worked with the common vegetative form of the pathogenic entamoebae, and so overlooked cyst formation, and that the small spores of *histolytica* in reality had nothing to do with multiplication, but were structures unrelated to the entamoebae. He considers that if Schaudinn were correct in naming the pathogenic species *Entamoeba histolytica,* the name *Entamoeba tetragena* is no longer required, even though Schaudinn were mistaken in his conception of the life cycle. Wenyon could confirm in every essential detail the observations of Viereck and of Hartmann on the pathogenic entamoebae, but could not confirm the life cycle of *histolytica* as described by Schaudinn.

The author, by artificially infecting cats, carried an infection through four passages without noting any diminution of vitality. He states that but for an accident he could have carried the infection further.

He is of the opinion that the thickness of the pseudopodia, to which has been attributed the property of penetration of the tissues by the entamoebae, has nothing to do with the entrance of the organisms into the glands and submucosa It appears to him that such entrance is due to some toxic substance which so weakens the epithelium of the tissue cells that the pathogenic entamoebae force their way through before the cells are disintegrated.

Darling (1913) notes that since there is no difference between the morphology of other pathogenic organisms found in the Canal Zone, and those found elsewhere, it is unlikely that the entamoebae found here differ in species or otherwise from those found in other countries. The pathology of amoebic dysentery is the same here as elsewhere. He is of the opinion that there is but one pathogenic organism, *E. tetragena*, since he could not confirm Schaudinn's findings as to the multiplication of *histolytica*. He notes that although the *histolytica* vegetative type is found here, animal inoculation demonstrates that this type is identical with *tetragena*.

Darling further notes (and his are the first observations, to my knowledge, on this point) that cysts are viable only for the first day or two after being passed. He describes the decrease in number of the cysts, when these are placed in moist chambers, in proportion to the time since they were passed. He believes that infection in man is brought about by the ingestion of a small amount of faecal matter containing a great number of cysts within forty-eight hours after these have been passed. The importance of freeing carriers from infection, and the routine examination of stools, is thus demonstrated.

There are in English two very good books on the entamoebae and on amoebic dysentery. These are by Brown (1910) and by Craig (1911). They will be found very useful to anyone taking up the study of these organisms, but lack of space prevents a further notice of them. An excellent history of amoebic dysentery is to be found in each of these.

In a recent paper of the greatest importance Craig (1913) revises his former opinion that *histolytica* and *tetragena* are separate species, and states his conviction that they are identical.

After a careful review of the literature, in which the various points of interest in the development of our knowledge regarding the entamoebae are clearly set forth, the writer gives the reasons for his present belief.

Until very recently it had not been shown that the process of reproduction by 'gemmation' and 'spore formation,' described by Schaudinn as characteristic for *E. histolytica* and confirmed by Craig, occurred also in *E tetragena*; nor had it been satisfactorily demonstrated that infections with *E. histolytica* went on to the formation of four-nucleated cysts. Darling (1913*c*), however, has lately demonstrated that in kittens artificially infected with *E. tetragena*, forms identical with those described by Schaudinn for budding and spore-formation in *histolytica* were produced, and he regarded these forms as degenerative. Darling found the 'budding' forms in wet-fixed and hematoxylin stained preparations, which disproves the statements made by some authorities that Craig's results could be obtained only after air dried and Romanowsky stained smears.

Craig then points out that in cases previously studied by him the dysentery was very severe and intractable, so that cyst formation did not take place. All of the said cases were on treatment with rectal irrigations of various drugs, and it was in some of these, although infrequently, that the so-called budding forms were found. Recent work and the study of new material, however, has convinced Craig that the 'budding' is not a reproductive, but a degenerative process.

In the new material Craig found typical *histolytica* forms going on to encystment, associated at the same time with entamoebae with nuclei of the *tetragena* type. In one of the preparations, made from a cold stool, 'budding' was observed in many of the entamoebae.

Notwithstanding the claims made by some authorities that Schaudinn's *E. histolytica* was described from degenerating forms of *E. tetragena*, and so is a spurious species, Craig insists on its validity as a well-determined phase in the life cycle of the pathogenic entamoebae of man. He refers to James' (1912*b*) statement that the *histolytica* type of nucleus is found in infections with very acute symptoms, while the *tetragena* type is found in more chronic infections. It was with the more chronic infections that the German observers, notably Hartmann and Viereck, worked, but the degenerative forms described by the former should not be mistaken for the healthy entamoebae with *histolytica* nuclei found in acute dysentery.

Craig then classifies the relation of the types of nuclei to the clinical symptoms as follows:—

'1. Entamoebae presenting the *histolytica* type of nucleus are most frequently found in patients presenting the most severe symptoms of entamoebic dysentery. This type of nucleus appears to be characteristic of *E. histolytica* when it is undergoing rapid multiplication by simple division, for no cysts are observed in these cases. That this type of nucleus is not degenerative, as claimed by Hartmann, is proved by the fact that such entamoebae occur most frequently in the worst cases of acute dysentery, and even though treatment is being administered, the entamoebae showing this type of nucleus differ entirely in appearance from those showing real degenerative changes brought about by the treatment. In addition, typical dividing forms are generally observed, snowing this same type of nucleus.

'2. Entamoebae presenting the typical *tetragena* type of nucleus occur most frequently in cases presenting slight dysenteric symptoms, and this type of nucleus appears to be characteristic of *E. histolytica* (Craig writes of the species as *E. histolytica*) when it is undergoing simple division preparatory to the formation of the small generation which produces cysts.

'3. Entamoebae reduced in size, and presenting generally the *tetragena* type of nucleus, although an intermediate type between this and *histolytica* is frequently observed, occur most frequently in cases of long duration (chronic dysentery) in which the symptoms are practically subsiding. This type is apparently characteristic of *E. histolytica* preparatory to the formation of cysts.

'4. The four-nucleated cysts occur most frequently in cases which have apparently recovered, and in which the stools are semi-formed or formed. These cysts are characteristic of *E. histolytica*, but occur only in a small proportion of cases, as shown by Wenyon, James, Ornstein, and others, and do not appear in acute infections, as shown by their absence in man and in fatal infections in kittens.*

---

* In order to obviate what may appear to be a discrepancy between this statement of Dr. Craig's and my own statements made elsewhere in this work, I may state that in infections observed by me in which the *bistolytica* type of nucleus predominated, encystment was very seldom encountered; but in all infections in which the type of nucleus was of the *tetragena* variety, encystment followed in a short time. The total of primary acute cases of amoebic dysentery in the Canal Zone in the past two years, showing the *bistolytica* type of nucleus in the entamoebae, was in excess of the chronic, sub-acute, and latent cases in which the *tetragena* type of nucleus was found.—W. M. J.

'5. Entamoebae presenting appearances that have been interpreted as reproduction by "budding" or "spore formation" occur most frequently in cases presenting subacute symptoms of dysentery. Such forms are associated with entamoebae showing the *histolytica* type of nucleus, and occur in cases in which the entamoebae are undergoing rapid multiplication by simple division. Most of the patients were receiving treatment by rectal irrigations of quinine. Similar forms, much smaller in size, are also found in association with cysts, and are not infrequently observed in patients who have apparently recovered. I believe that these forms are degenerative, and may be produced by rapid exhaustion of the race, as suggested by Darling, or by the effect of remedial agents, as quinine. Personally I am of the opinion that both causes may operate in producing these so-called "budding" forms of *E. histolytica*.'

Craig then presents a life cycle for *E. histolytica*, based on the following data:—

1. In the vegetative stage two distinct types of nuclei are found, the *histolytica* associated with acute dysentery, and the *tetragena* associated with more chronic cases. Between the two all degrees of variation may be encountered.

2. *Precystic Stage.* In this the entamoebae decrease in size, and resemble very closely those described by Elmassian for *E. minuta.* The nucleus is intermediate in type between *histolytica* and *tetragena.*

3. *Cystic Stage.* The cysts are from 7 to 20μ in diameter, the average being about 15μ. They resemble exactly those described as characteristic of *E. minuta.*

In conclusion, Craig gives to Walker the credit for being the first definitely to state the identity between *histolytica* and *tetragena.* The name *tetragena* must be dropped, as having no standing, and either *histolytica* be substituted or *coli* or *dysenteriae*, depending on whether priority be awarded to Schaudinn, or to Lösch, or to Councilman and Lafleur.

Craig emphasizes the difficulty of a proper classification as long as protozoologists insist on placing so much stress on minute morphological details, in so variable a structure as the nucleus, without regard to a study of the complete cycle.

In his latest contribution Whitmore (1913) has presented a paper of the greatest interest. In the review of the literature he notes in detail the more recent progress in the study of the entamoebae of man, and gives a concise and accurate account of the work of Viereck, Hartmann, and Huber, in describing the morphology of *E. tetragena*. Whitmore's close association with Hartmann in the latter's studies on *tetragena* and *coli* enables him to present accurately Hartmann's views.

In discussing the proper nomenclature for the pathogenic species Whitmore gives the recent opinions of well-known authorities, and states that in view of the work done in the past few years, the identity of *histolytica* and *tetragena* has been established. The name *tetragena* should be dropped as a species name. He quotes Hartmann with respect to *histolytica* as follows:—

'The dysentery amoebae are handled as two separate species. This is done only on the basis of the preparations of one of Schaudinn's cases, and on his infection experiment. If in this case the identity with *E. tetragena* can be established, which I (Hartmann) consider very probable, then the name *E. tetragena*, according to the rules of nomenclature, will have to be eliminated. In this case, following Doflein's (1911) suggestion, I propose the old name *Entamoeba dysenteriae*, Councilman and Lafleur (1893), although these authors gave no recognizable morphological characteristic of the amoeba. Then one might speak of the *tetragena*-form as the typical form, and of the *histolytica*-form as the degenerative form.'

Whitmore revises the opinion quoted from the joint contribution of Hartmann and himself (1912) as to the identity of *coli* and *minuta*, and gives his opinion of the latter species as follows:—

'I have recently had the opportunity to examine some material sent to me as *E. minuta*, and I can unhesitatingly say that this material is *E. tetragena*.

'Since studying this material, I have changed my opinion from that expressed by Hartmann and Whitmore (1912) that *E. minuta* is identical with *E. coli*. I believe that *E. minuta* was described mainly from the encystment stage of *E. tetragena*, and probably also from *E. coli*.'

The rest of his paper is concerned principally with the physiology of the entamoebae, and their relation to dysentery, and some of the points raised are discussed by me in Part III.

PART II

MORPHOLOGY OF THE ENTAMOEBAE FOUND IN MAN IN THE CANAL ZONE

I. INTRODUCTION

This study of the entamoebae parasitic in man was taken up two years ago. It had for its object not only the classification of the diverse types met with here, but also the relation between these types and the clinical forms of amoebic dysentery. I have attempted also to demonstrate a method of laboratory identification of the entamoebae that does not demand too great an expert training.

Recent literature on trypanosomiasis emphasizes the difficulty of identification when that differentiation is based on morphological criteria alone. Size, shape, position of the nuclei, motility and other data pertaining to morphology are used to determine species; but in default of an exact knowledge of the life cycle of each species a unanimity of opinion does not prevail. How far the morphological criteria are peculiar to species, and how far such criteria are the result of external conditions, does not seem to have been determined. As Calkins (1909) points out: 'The modifications brought about by these several conditions (changes in the blood; in external conditions; transfer from the blood of mammals to an insect's digestive tract, or to cultures) have been variously interpreted as sexual differences, as resting phases, and the like, while so-called latent bodies and encysted forms have been found in some cases. P. 257.'

Yet the phases of trypanosomes met with in man serve at once for an identification of a very grave and highly fatal disease, and I believe that it is of less importance to determine the species in such a case than it is to find the pathogenic organism.

A similar lack of unanimity of opinion as to the determination of species is plainly manifest in the more limited literature of the *Amoebae*, or, as they are termed generically, the *Entamoebae*, of the human intestinal tract. The problem of determination of species in these organisms is quite as difficult as that of human trypanosomiasis; because there is a non-pathogenic species of entamoeba, and certain phases of the pathogenic species may not give rise to symptoms at the time of examination, whereas the finding of only one trypanosome in the peripheral blood is a certain indication of a pathogenic infection, and symptoms are nearly always present. Moreover, the entamoebae have not as yet been cultivated.

And as in the study of the trypanosomes, criteria accepted by some as characteristic of species are rejected by others as artefacts, degeneration forms, or variations from type, so it is in the study of the entamoebae of men, as was shown in Part I. The trained protozoologist will often place on his own findings an interpretation quite different from that held by one of lesser experience, although the facts recorded by the latter may be entirely correct from the morphological point of view.

Unfortunately, the busy physician in the tropics, as elsewhere, has but little time for the study of protozoology, important as this is. A subject with one of the largest literature lists of any branch of science demands one's exclusive efforts, and an accurate knowledge of the pathogenic protozoa alone is beyond the attainment of the practising physician for many years, unless he is so fortunate as to be able to devote time to such a study. His opportunities for research are limited by the demands of his practice. Yet no conscientious physician will attempt to practise in tropical countries without frequent use of the microscope. For the interpretation of his findings, therefore, whether deduced from individual study solely, or whether supplemented by a course in one of the Schools of Tropical Medicine, he is largely dependent on the observations of those who have had time and opportunity for research.

If there were that unanimity of opinion among those who have studied the entamoebae of man which would permit one who encounters these organisms occasionally to make a positive identification, there would be no necessity for this contribution. The difficulty of identification does not lie so much in the correct determination of the pathogenic species when these are associated with acute amoebic dysentery; this stage of the life cycle is distinguished without much difficulty from the non-pathogenic *coli* in either the *histolytica* or the *tetragena* type, in my experience.

But those phases of the pathogenic entamoebae encountered in the interval between attacks, when no dysenteric symptoms are present, and blood, pus, and mucus are absent from the stools, are not so easily determined, and may be considered as stages in the cycle of *E. coli*, with consequent failure, sometimes disastrous to the patient, to institute proper treatment.

For instance, an entamoeba found here in latent intervals, or before any symptoms of dysentery have appeared, has been described most thoroughly by Elmassian (1909) with the name of *E. minuta*. This organism differs in morphology and in its observed life cycle from *tetragena* as described by Hartmann (1912). Hartmann, however, as noted in the abstract of his paper in Part I, refers to *minuta* as identical with *E. coli*. But, as will be seen later, the *minuta* type as found here has nothing in common with *E. coli*, except that its life cycle is on the same general plan. *E. minuta*, as observed in the Canal Zone, is associated with a very mild type of dysentery, and for the most part is found in the intervals between relapses. Had I diagnosed infections of the *minuta* type as *E. coli* infections, or had I failed to recognize the former when dysenteric symptoms were absent but other indications of intestinal disturbance were present, the subsequent treatment would not have effected a removal of the offending organism.

Similar experiences have led me to the belief that at present too great a reliance should not be placed on the opinions of any one authority, no matter how high his reputation. Such opinion should be given the most careful attention, for it is highly improbable that observations based on expert study are morphologically incorrect. But when authorities differ so pointedly among themselves with respect to proper interpretations, it is not unreasonable to give expression to one's own views, if these are founded on conscientious study and experiment. I make no claim whatever to an expert knowledge of protozoology; the observations and conclusions set forth in this paper are those any physician might make who has had experience in microscopy and tropical medicine. I have had, however, opportunity to devote myself almost exclusively to this study for the past year, and I can claim some familiarity with the technique and methods of observation used by those more expert than myself.

II. MATERIAL

The material on which this communication is based was obtained from about sixty cases of amoebic dysentery and infections with pathogenic entamoebae, admitted in the past two years to the wards of Ancon Hospital. In several of these infections *Entamoeba coli* was present also, giving an opportunity for comparative study in mixed infections. Over forty of these cases were studied by methods of wet fixation, while in all observations were made on the living organisms.

Entamoeba coli was studied also in several cases of single infection, particularly in some in which the infection had persisted for years. These latter I could observe through the courtesy of Dr. John M. Lawler, Superintendent of the Insane Asylum connected with Ancon Hospital.

For many of the specimens dry fixation, or fixation in absolute alcohol, followed by a modification of the Romanowsky stain, was used.

I am indebted for the opportunity to study many of these cases to my colleagues on the medical side of Ancon Hospital; in particular to Drs. F. F. Monroe, Walter Baetz, L. M. Drennan, and H. R. Carter, Jr. Dr. Carter was associated with me on my section, and many of these cases were first diagnosed by him.

I have a section of an amoebic ulcer of the gut, which came from the material used by Councilman and Lafleur in the preparation of their notable monograph (1891). This was given to me by Dr. Gary N. Calkins, of Columbia University, with several preparations of culture amoebae. Other sections of liver abscess and amoebic ulcers were given to me by Dr. R. E. Hill, Pathologist to Colon Hospital, and Dr. H. C. Clark, Pathologist to Ancon Hospital.

I have also specimens of *E. minuta*, sent by Dr. Elmassian, of Paris, and of *E. tetragena*, and a free living species, sent by Dr. Edouard Chatton, of the Institut Pasteur, Paris. From Dr. C. M. Wenyon, of the London School of Tropical Medicine, I received some extremely valuable slides, and Major Eugene R. Whitmore, Medical Corps, U.S. Army, lent me an excellent preparation of *E. tetragena* in the vegetative stage, from the material brought by him from Manila to Berlin, and there studied by Dr. Hartmann and himself.

I am indebted to Dr. M. Elmassian for drawings from my specimens presented with this article (Pl. XV, figs. 100-116), and to Dr. A. Gauducheau, of Tonkin, French Indo-China, for pictures of the common dysentery entamoeba of that country (Pl. XVI, fig. 123).

From time to time I have sent specimens of my material to different authorities, and I am especially indebted to Dr. M. Elmassian, Dr. Gary N. Calkins, Dr. Gustave Mann, of Tulane University, New Orleans, Captain C. F. Craig, M.D., and Major E. T. Whitmore, M.D., Medical Corps, United States Army, for comment and opinion with regard to these. I am indebted also to Dr. S. T. Darling, Chief of Board of Health Laboratory, Ancon Hospital, for the opportunity to observe his specimens from artificially infected cats, and the results of his experiments with cysts in moist chambers.

I wish to take this opportunity to express my warmest thanks to all of these gentlemen for their kindness and assistance.

Several methods of fixation and staining were used on this material, and an account of these is given in Part IV.

III. MORPHOLOGY

Many protozoologists have pointed out the importance of viewing the entire cycle of a protozoon before any attempt at classification is made. This is because the organisms vary so much in different stages that observers who have studied only certain phases of the cycle will disagree as to the determination of species.

Calkins (1909) has given an excellent account of the life-cycle in general as applied to the protozoa. He describes three phases: youth, maturity, and old age. In youth the organisms are at the period of greatest activity and pathogenicity, and there are fewer variations from type. When the organisms reach maturity there are changes in the cytoplasm, plastic in kind; the nuclei are more definite and typical; and conjugation takes place. Old age is shown by marked degenerative changes in the cytoplasm, such as vacuolization and granulation, and the nuclei also degenerate. Unless there is in some way a rejuvenation of vitality, the cycle will end here, to be taken up later, under suitable conditions, by the resistant forms which have been developed earlier for this purpose.

These facts have a very important bearing on any study of the entamoebae of man, and particularly on a morphological study. They aid in determining whether the morphological differences are due to plurality of species, or to phases of the life-cycle; and assist also in making clear what may be obscured by conflicting opinions.

I shall present first, from the point of view of morphology, a study of the entamoebae parasitic in man in the Canal Zone, and later shall endeavour to make a classification according to the previously determined species.

Entamoebae associated with acute dysentery. Dr. W. E. Deeks has shown how many forms of dysentery other than those of amoebic origin are to be found in the Canal Zone. It is of highest importance, then, to distinguish between the harmless *E. coli*, which is at times associated with these non-amoebic dysenteries, and the pathological varieties which are the true etiological factors in amoebic dysentery.

In the past few years, owing perhaps to improved methods of sanitation, infection with *Entamoeba coli* has markedly decreased, so that at present it is not very often associated here with acute dysentery, either of the amoebic or non-amoebic type. In heavily infected communities, however, *E. coli* not infrequently is found associated with non-amoebic dysentery. In such an instance a knowledge of its morphology and life-cycle is of greatest value.

Three types of pathogenic entamoebae have been found associated with acute dysentery in the Canal Zone. That most commonly found corresponds in all details to the organism described by Schaudinn and by Craig as the vegetative stage of *Entamoeba histolytica*. This I shall designate as Type A. Much less common in acute dysentery is the organism described by Hartmann as the vegetative stage of *Entamoeba tetragena*, which I shall designate as Type B. Three times I have encountered an organism which, in fresh preparations, is identical with type A, but which, when wet fixed and stained, shows characteristics intermediate between types A and B. This last I shall designate as Type C. All three of these types refer solely, for the present, to the vegetative organisms associated with tenesmus, frequent stools, and the passage of blood, pus, and mucus.

Type A

(*Entamoeba histolytica*, vegetative stage, as described by Schaudinn and by Craig)

Entamoebae of this type are for the most part large, the size being from 30-60 μ. Some are larger than this, and I have measured entamoebae that in extension were 80-90 μ. Very exceptionally the size may be from 10 to 30 μ in organisms otherwise identical with this type. In preparations examined immediately after the stool is passed, active motion, as a rule progressive, is characteristic of these organisms. *Entamoeba coli* at no time shows such activity, although the rapid flow of endoplasm into ectoplasm, sometimes observed, will simulate the more active motion of the pathogenic species. It should be carefully noted that motility in all entamoebae is relative; unless one has observed the characteristic rapid movement of the pathogenic species, one may easily mistake lesser degrees for it. But once seen, this movement will immediately identify a pathogenic organism. Unfortunately such movement is often rapidly lost after the stool is passed, and is of service in positive identification only when stools can be examined at once.

The various body cells that are found in stools of acute dysentery, amoebic or otherwise, are not infrequently taken for 'non-motile' entamoebae, especially as certain of these cells often phagocytose erythrocytes. Although most of the pathogenic organisms lose their rapid movement, as noted, soon after the stool is passed, many of the entamoebae show motion of some kind for an hour or more at room temperature in the tropics. But so great is the resemblance in fresh preparations between entamoebae which have lost motility, and are in various stages of degeneration, and the body cells in the intestinal contents, that a diagnosis based on non-motile vegetative organisms is not justified, unless confirmed by stained specimens. Even in these, although wet fixed and stained, the cytoplasm of some entamoebae and some body cells is morphologically almost identical, and a study of nuclear structure is necessary to identification. When fresh preparations are being used for diagnosis, if there is doubt as to whether entamoebae or body cells are in question, another specimen should be procured. There need be no such uncertainty, however, in freshly passed specimens; in these many of the entamoebae, if present, will always show motility, and the characteristic active motility if the organisms are pathogenic, unless drugs such as quinine and thymol have been used for irrigations, or bismuth has been given by the mouth. Experienced observers have not infrequently mistaken body cells for entamoebae, not only in fresh preparations, but also in wet fixed and hematoxylin stained preparations, as has been demonstrated by subsequent criticism.

It is in the entamoebae associated with acute amoebic dysentery that the often described differentiation between endoplasm and ectoplasm has the highest value. In this stage of the disease the entamoebae present an ectoplasm highly refractive, homogeneous, and in sharp contrast to the alveolar and less refractive endoplasm. *Entamoeba coli* when in motion frequently shows a distinct ectoplasm, but this is never as refractive as that of the pathogenic species in acute dysentery. The distinction between ectoplasm and endoplasm, like that between the degrees of motility, is relative, and the criterion is the differentiation shown in the actively motile pathogenic organisms. If one has never seen this, one may at times easily take the lesser differentiation in *coli* for the greater in the pathogenic species.

The pathogenic species are actively phagocytic for erythrocytes. Sometimes, even when blood is plentiful in the stools, many organisms will not contain erythrocytes, but more often these are observed in the endoplasm, not infrequently in enormous numbers, so that the interior of the entamoeba is literally stuffed. In mixed infections I have often observed erythrocytes in varying quantities in the interior of the pathogenic organisms, but I found them rarely within the coincident *Entamoeba coli*, and then only one or two at a time. Absence of erythrocytes from the endoplasm is not of any particular value in differentiation, but their presence in any quantity is a certain indication of a pathogenic species.

Pathogenic entamoebae do not to any degree phagocytose bacteria, intestinal protozoa, and stool detritus. This is, as Hartmann (1909) notes, a well-defined criterion of differentiation between them and *E. coli*, which latter organism at times is actively phagocytic for bacteria and protozoa. Phagocyted inclusions other than erythrocytes and occasional cell nuclei are probably accidental, and I have yet to see a pathogenic entamoeba stuffed with an apparently selected portion of the intestinal contents, except erythrocytes, such as may be seen in *E. coli* (figs. 63, 152).

The presence of vacuoles in healthy pathogenic organisms depends largely on the included food material. If none of this has been phagocyted, the cytoplasm is alveolar in most cases, although some of the alveoli will very closely simulate vacuoles, On the other hand, the inclusions are enclosed in very well-defined vacuoles with clear-cut margins (figs. 1, 2, 3), anl are best demonstrated in wet fixed preparations. When many erythrocytes have been phagocyted, the entamoeba will appear to be made up almost entirely of vacuoles of different sizes. In fresh preparations degenerating organisms almost always show large vacuoles, and not infrequently a single vacuole will take up most of the cytoplasm. The vacuoles are never contractile, as in the free living species, as has been shown by many writers.

As first noted by Craig (1905), in specimens from bloody stools the cytoplasm of the pathogenic species is often of a well-defined light-green colour, quite different from the grey tint of *E. coli.* This appearance, when present, is quite characteristic, but its determination seems to depend on the personal equation of the observer. Craig attributes this colour to a solution of the hemoglobin of the phagocyted erythrocytes. Hartmann does not mention it, nor do his drawings of the living entamoebae show it. Brown (1910) states that 'the colour varies with the food ingested, and with the number of granules; but, in ordinary circumstances, the endoplasm of *E. histolytica* has a pale, greenish-yellow tint—that of *E. coli* being brownish grey.' Darling (1912*c*) states that he has not observed this greenish tint, with either natural or artificial illumination. Working under the same conditions as this last, I can state that I have observed the characteristic colour very frequently, provided there is plenty of blood in the stools. It may be, perhaps, due to refraction, instead of solution of hemoglobin within the entamoeba.

The included erythrocytes are orange coloured, after Hastings' and Giemsa's, and Mann's stains, and of all sizes (figs. 124-126). It is to be noted that, although they may be but a fraction of their normal size, they stain as do the normal erythrocytes free in faeces, or even overstain. Darling (1912*c*) is of the opinion that the erythrocytes disappear rather by 'condensation and erosion,' than by solution. Whatever may be their fate, or their relation to the physiology of the organisms, it is certain that as far as can be demonstrated by staining reactions they gradually disappear within the cytoplasm of the entamoebae, and apparently the vacuoles are the causative factors in this disappearance. If the erythrocytes have any important part to play in the nutrition of the entamoebae, it is during the stage of acute dysentery, for in later phases of the cycle they are absent from the stools and from the cytoplasm of the entamoebae as well. There is some difference of opinion in regard to the ingestion and fate of the erythrocytes. Brown (1910) states that he has never observed actual phagocytosis of an erythrocyte, and, so far as specimens of entamoebae under the microscope are concerned, doubts whether absorption takes place. On the other hand Craig (1911) states that he has frequently observed experimental ingestion of the erythrocytes, and has watched these slowly disappear within the entamoeba, the organism at the same time taking on a greenish colour. Personally, I have never witnessed ingestion, nor have I seen disintegration of the erythrocytes within the living organism, neither have I given as much time to the study of this particular phase as has Dr. Craig.

The inclusion of erythrocytes in the pathogenic species may follow in the same manner as does that of various protozoa and bacteria by *E. coli*. Phagocytosis of divers intestinal inhabitants by the latter species does not appear to be necessary to life, since the entamoebae thrive equally as well in the absence of some of these as in their presence. If any particular species of bacterium is necessary to the existence of *E. coli*, it has not as yet been demonstrated. In some phases of the cycle of *E. coli*, as will be shown, the entamoebae are not at all selective, while in others a certain organism will be selected almost to the exclusion of others. Unless the ingestion and digestion of erythrocytes is essential to the development and maintenance of that phase of the life cycle during which the pathogenic species manifest their most noxious effects, I see no *a priori* reason why the inclusion of red blood cells by organisms of the *histolytica* and *tetragena* types is any more essential to the life of these than is the phagocytosis of a *Trichomonas* by *E. coli.*

I have never witnessed actual phagocytosis by any species of the entamoebae. Since, however, the process obviously takes place in the large intestine, it may very well occur, if conditions are favourable, under the microscope as well. The explanation of why phagocytosis occurs, and of its relation to the physiology of the organisms, is only one of the many interesting, but unsolved problems in the study of the entamoebae.

The above observations, although classed under the description of Type A, hold for the vegetative forms of all three types when associated with acute amoebic dysentery.

In fresh preparations, the nucleus of a Type A organism is made out with difficulty, and often cannot be seen at all. It is eccentric, poor in chromatin, and its shape is easily altered by internal pressure, such as when it is passing between erythrocytes or included particles, or when it is pressed against the ectoplasm. As described by Schaudinn and by Craig, the chromatin consists of a few scanty grains, arranged in an irregular circle or ellipse, and the very delicate membrane can be made out only under the most favourable conditions of observation.

Although not seldom specimens of *Entamoeba coli* lack a visible nucleus, this is easily observed in most organisms of that species. It has abundant chromatin, arranged in blocks and granules, easily discernible, under a well defined double contoured membrane. Often a central refractive granule, the karyosome, is seen. Except in certain phases of division, this nucleus retains its shape as it moves about in the cytoplasmic currents, and pushes aside the vacuoles and food contents of the endoplasm. For the most part it is placed rather towards the centre of the entamoeba.

In wet-fixed and stained specimens, after all the methods I have used, entamoebae of Type A show a fairly constant morphology. The ectoplasm is not often differentiated from the endoplasm, but sometimes pseudopodia are seen, which are finely granular. The fixative used has a marked influence on nuclear and cytological appearances. With Duboscq's fluid, the cytoplasm and nuclear structure are more granular than after Zenker's and Schaudinn's fixatives. The well defined vacuoles, frequently of considerable size, so often seen in the endoplasm of *Entamoeba coli*, are not often present except in degenerating organisms. Hematoxylin staining for the most part shows as included products only erythrocytes and nuclei, or remains of these, of other cells. It has been disputed as to whether the entamoebae phagocyte nuclei of other cells. My work has convinced me that they do, for after Mann's stain, or after dry fixation and Hastings' and Giemsa's stains, the phagocyted nuclei show distinctly, and are easily identified. With Mann's stain the cytoplasm is beautifully demonstrated (figs. 145, 146).

The nuclear appearances are quite characteristic. If the nucleus is under any pressure it appears distorted, but otherwise it is round. The nuclear membrane is best shown by Mann's stain, as monochrome stains are likely to deceive by showing not infrequently the condensation of peripheral chromatin as an apparently thickened membrane. After Mann's stain the nuclear membrane shows most frequently as a delicate circle of blue, against which the chromatin is contrasted in shades of pink and orange (figs. 145-148). The colour reactions within the nucleus are in all probability closely related to the physiology of the organism. On or under this membrane the chromatin is arranged in scanty blocks or granules, sometimes as a thin band, which may be crescent shaped, or, as the French very well express it, *en calotte*. In the centre is a small karyosome, in the interior of which sometimes a tiny centriole can be observed. This is very well shown in figs. 100-103 by Dr. M. Elmassian, while the various aspects of the distribution of the chromatin is beautifully demonstrated in the drawings of Dr. A. Gauducheau (fig. 123). The space between the karyosome and nuclear membrane, the 'Aussenkern,' shows sometimes a delicate network, which does not take the stain well. On this network, particularly at the nodes, are fine particles of chromatin (fig. 102). With Mann's stain this portion of the nucleus takes a different tint from the karyosome and the peripheral chromatin (figs. 145, 146). After iron hematoxylin the *Aussenkern* often stains merely as an ill-defined network, or diffusely. (figs. 1, 2, 3, 100-103). This part of the nucleus very probably is composed of achromatinic substance, such as linin, and stains after the so-called nuclear dyes in proportion to the amount of nucleinic acid present. The peripheral chromatin and the centriole react identically to all of my reagents, while the karyosome varies decidedly in intensity of staining. According to Minchin (1912) the karyosome is composed of varying amounts of chromatin and plastin, and stains intensely in proportion to the amount of the former. I shall discuss this further under the next heading.

Cyclical changes in the karyosome, such as are described by Hartmann (1912) for *tetragena* cannot be ascertained with any distinctness in this type. At the most, there is a deeply staining karyosome, in which the centriole cannot be distinguished; or a fainter staining karyosome with a very definite centriole (figs. 1-3, 100-103). Sometimes a faint ring of dimly stained substance can be made out between the karyosome and the nuclear membrane (fig. 1). None of these appearances, however, have any definite correlation with the amount of chromatin in the *Aussenkern*, as in the type next to be considered.

Such a nucleus, with a delicate membrane, scanty chromatin, and a minute karyosome, or a small centriole surrounded by a delicate or ill-defined karyosome, corresponds very closely to that described by Schaudinn, Craig, Gauducheau (1912) and others for *Entamoebae histolytica* (figs. 1-3, 100-103, 123, 145-146).

After dry fixation, or fixation after drying in absolute methyl alcohol, and staining with Hastings' and Giemsa, the nucleus is a light blue, with a darker periphery. Proper differentiation (see Part IV) often shows a delicate network of blue threads, inclosing unstained spaces at times (fig. 126). This appearance has been noted by Darling (1912*a*). The red or purple staining substance, commonly called chromatin, but which I shall designate as erythrochromatin, is arranged in fine threads and bars, scattered irregularly over the blue network (figs. 124-126). It is not certain if this erythrochromatin is the same as the hematoxylin-stained substance commonly called chromatin, but it is intimately associated with the latter; because in the cysts the erythrochromatin appears in rings, corresponding to the hematoxylin-stained nuclei. In dry-fixed and hematoxylin-stained specimens, however, the chromatin appears as a mass of deeply-stained, irregular granules, and this appearance is not duplicated in red after Hastings and Giemsa. Darling (1912*c*) believes that there are two substances in what is commonly called chromatin; one taking the red of a Romanowsky stain, and the other the blue or black of haematoxylin, and this is also my own opinion, as expressed in a former paper.

I have not had success in staining with a Romanowsky method after wet fixation, and so cannot compare the difference between wet and dry fixation after such a stain.

However, the nuclear morphology is quite definite after dry fixation and staining with Hastings and Giemsa (see figs. 124-126), and can be easily differentiated from that of *Entamoeba coli*, as will be shown subsequently.

With this method the endoplasm stains in various shades of blue, the tint depending very likely on the physiology of the pathology of the organism. Degenerating entamoebae stain a very pale blue. The endoplasm is granular, the granules being darker than the ground substance. The erythrocytes are light orange. Very frequently masses of ill-defined, red-staining substance are met with in the endoplasm (figs. 124-126). Analogues of these are not seen after wet fixation and hematoxylin staining, and these masses may be either stool detritus which has adhered to the organism in drying, or they may be products of digestion which do not stain after hematoxylin, as Gauducheau (1912*a*) notes. There is but little doubt in my mind that these masses have been taken for distributed chromatin by others, who have claimed to follow the so-called sporulation, as Darling (1912*c*) has pointed out.

The ectoplasm is more frequently seen than by wet fixation, and as a rule stains a darker blue than the endoplasm. It is very finely granular, almost homogeneous. But the dark blue of the masses of granules often massed at the periphery should not be mistaken for ectoplasm (fig. 125, ectoplasm at top).

An entamoeba of Type A, as it appears after dry fixation and staining with Hastings and Giemsa, is illustrated in fig. 125 (126-127 are Type B). This entamoeba corresponds exactly to those pictured by Craig after the same method as *Entamoeba histolytica*.

Type B

(*Entamoeba tetragena*, vegetative stage, as described by Hartmann.)

This type of pathogenic entamoebae, as found in the Canal Zone, corresponds in every essential to that described by Hartmann (1912) as the vegetative stage of *Entamoeba tetragena*.

The only difference morphologically between Types A and B is in nuclear structure and appearance. In the living organisms the characteristics of motility, differentiation between ectoplasm and endoplasm, and the structure of these, are identical for the two.

The nucleus of Type B, however, is clearly evident in most instances in the fresh specimen, and I have nothing to add to Hartmann's (1912) description: 'It shows itself as a well-rounded, globular vesicle, plainly set off from the cytoplasm by a stout, double contoured membrane. The finer structure of the nucleus can be clearly recognized. Most nuclei show in the middle a little karyosome, which is surrounded by a clear, structureless zone. Between this zone and the nuclear membrane is a honeycomb linin network, on which the granules of chromatin are distributed.'

This appearance varies with the cyclical changes (see Part I, abstract of Hartmann's paper on *tetragena*); so that sometimes the karyosome is prominent, with or without blocks of chromatin under the nuclear membrane, and at others there is only a small central dot. The entire nucleus appears to be more refractive than the endoplasm, so that even when the blocks of chromatin are not present it is easily seen. As in other species, visibility of the nucleus varies, and the above account refers to what is observed in the majority of the individuals.

In the living organism the nucleus is very similar to that of *Entamoeba coli*, and, personally, I could never satisfy myself as to any constant points of differentiation. The difference in cytoplasmic structure between the two is well marked, however, and will be referred to in the description of *E. coli*.

After wet fixation and staining, the cyclical changes of the karyosome, as described by Hartmann, and quoted in detail in the abstract of his work in Part I, are plainly seen, particularly after staining with phospho-tungstic acid hematin. In the same preparation many of the entamoebae show similar stages of the cycle, so that all of the changes are not plentiful at any one period. In one of my preparations nearly all of the entamoebae showed a definite centriole, surrounded by a clear zone; a karyosome of considerable size, and very definite; and large blocks of chromatin under the nuclear membrane (figs. 24, 25). The next day many of the organisms showed instead of the well-defined and circular karyosome and the large blocks of chromatin, many small granules of chromatin in the *Aussenkern* and under the membrane. There was no very definite distinction between centriole and karyosome, and the organisms resembled very closely those of Type A, except that the karyosomes were better defined (fig. 21). Sometimes the karyosome appears as a collection of minute chromatinic granules surrounding a small centriole, and with scanty chromatin elsewhere in the nucleus. Such an appearance may be considered, perhaps, as intermediate between Types A and B, and is very well shown in Dr. Elmassian's drawing (fig. 108).

One of the differential criteria between the *tetragena* (Type B) and the *histolytica* (Type A) nuclei constantly referred to is the thicker nuclear membrane of the former. While it is true that in fresh preparations the membrane of the Type B nucleus is more often visible than that of the Type A, to me this peculiarity appears due more to a difference in chemical structure than to actual thickening of the membrane. Although it is true that when most of the nuclei are visible in fresh preparations, stained preparations will show abundant chromatin, I would not assert that the contrary is also true, for I have examined many preparations of both the pathogenic and the non-pathogenic species in which the nuclei were indistinct in the fresh state, but showed abundant chromatin when stained. Also, frequently the nuclei of the cysts can be made out only with difficulty or not at all in fresh preparations, when they show well enough in stained ones. A careful examination of either hematoxylin or Mann's stained preparations will show that the so-called thickening is due to condensation of chromatin under the membrane and not to a thickening of the membrane itself, also that the latter is a structure quite distinct from the chromatin. Although Hartmann (1912) refers to the *tetragena* type of nucleus as having a thicker membrane than that of the *histolytica* type, his own beautiful drawings give the impression that much of the thickening may very well be due to condensation of the chromatin. Compare Hartmann's figs. 4, 6, 20, with 8 and 9.

In most of my wet-fixed and stained preparations from Type B infections the membrane, when it could definitely be separated from the peripheral chromatin, was quite as delicate as in preparations from Type A infections. The morphological difference between the nuclei of the two types lies in the scantier chromatin, both in the karyosome and under the nuclear membrane in the Type A, and the occurrence of well-defined karyosomes with definite cyclical changes in the Type B nuclei. (Compare figs. 1, 2, and 3 with figs. 22-25*b*).

In Type B, after dry fixation and staining with Hastings and Giemsa, the nucleus is better defined than in Type A, and often of a darker blue. In nearly all of my specimens the erythrochromatin was arranged in a very well-defined, dark purple-coloured circle, occupying the position of the karyosome in wet-fixed specimens (figs. 125, 126). This arrangement of the chromatin is quite different from that found in *Entamoeba coli*, as will be shown later, and it is my belief that this method of staining will serve to differentiate the two. The staining reactions of the cytoplasm and included contents is the same as described for Type A, after any method of fixation and staining.

Type C will be taken up in the next section.

Hartmann (1912) states that he can affirm with great certainty that almost all cases of amoebic dysentery are due to *E. tetragena*, not to *E. histolytica*. By this he means that the organism described by him as *Entamoeba tetragena* is the common etiological factor in amoebic dysentery. The vegetative stage of this organism has been described above as Type B.

In my experience in the Canal Zone it is Type A, which corresponds to *Entamoeba histolytica*, that is commonly associated with *acute* amoebic dysentery, and not Type B. In the past eighteen months, in fact, since the beginning of this study, I have found Type B in five cases only of acute dysentery, while Type A has been found in all others.

It is not unusual to find in one type organisms of the other, as was noted by Walker (1911), but I have not observed in an untreated case a definite conversion of Type A to Type B, or the reverse. There is, however, evidence of a relationship between the two. For instance, in Type A the sum of the nuclear morphological characteristics is remarkably constant, and nuclei definitely resembling Type B are found infrequently. On the other hand, in infections with Type B, nuclei resembling those of Type A are found quite often; although the sum of the morphological characteristics of the nuclei of Type B, on the whole, presents well-defined qualities absent in Type A, such as the cycle of the karyosome. The deductions from these observations will be presented in Part III.

ENTAMOEBAE ASSOCIATED WITH MILD OR WITH LATENT DYSENTERY

Although it is not difficult to identify the pathogenic entamoebae when these are found associated with acute amoebic dysentery, the correct determination of organisms found in the intervals between acute attacks requires careful study and some experience. These latter are the so-called latent forms, and it is as important to be certain of these as it is to know the pathogenic types. As Darling (1912*b*) has pointed out, persons who harbour these latent forms are carriers of the disease, not persons with acute dysentery, for the development of latent forms is, in my experience, always followed by cyst formation. And, as I have noted, such forms should be recognized for the sake of the patient, to prevent relapse and the development of liver abscess. I have only twice observed cysts associated with the vegetative entamoebae of acute amoebic dysentery. Dr. Elmassian, in examining specimens from one of my Type B infections, found four-nucleated cysts in the same preparations as the large vegetative forms. Walker (1911), as noted, observed cysts and entamoebae of the *histolytica* type at the same time in two of his cases. Such findings, however, are not common, and very likely occur only at the beginning of a relapse. Perhaps, if a primary infection does not take on pathogenicity for some time, cysts may be found.

Until now, the cycle of a pathogenic entamoeba, covering the period from the symptoms of acute dysentery to apparent healing, has been described only by Walker (1911), Hartmann (1912), Darling (1912-13), James (1912*b*), and Craig (1913). Craig, as noted in Part I, now recognizes but one pathogenic species, and includes in the cycle of this all descriptions of the other pathogenic species. Elmassian (1909) described a complete cycle for *E. minuta*, but refers to the symptoms produced as diarrhoea and intestinal disturbance of a chronic kind rather than those of dysentery.

Schaudinn's account of the developmental phases of *Entamoeba histolytica* cannot be accepted without further confirmation. It is of interest, then, to note the important stages in the transformation of the large vegetative stages of *tetragena* to four-nucleated cysts. I do not believe that such transformation takes place in any individual organisms, but only after repeated division, so that with each division the entamoeba approaches in morphology nearer to the end stage. According to Hartmann:—

1. Before encystment, the entamoebae become smaller, and the chromidia appear. There is no description of endoplasm and ectoplasm, although from a previous statement I infer that the ectoplasm is less characteristic at this time.

2. In the normal vegetative forms the typical nucleus persists, but in some chromidia-forming individuals the nuclear membrane loses its distinctness, and is less plainly marked, as if it had become fluid. These nuclei lose their normal rounded shape, and are distorted by the plasma currents. Such appearances Hartmann describes as pertaining to the *histolytica* type, but they are not characteristic of what I term Type A. My figs. 5-9 show what I take to be the entamoebae described by Hartmann.

3. The organisms containing chromidia now go on to encystment. The entamoebae become rounded, and secrete a thin, sticky layer, from which develops first a single, and then a double membrane. The nucleus again shows the characteristics typical for *tetragena*.

4. Nuclear division follows, resulting in two nuclei with abundant chromatin. Each of these divides, so that the end stage is a four-nucleated cyst. To judge from Hartmann's pictures each of the four nuclei contains relatively abundant chromatin.

Generally speaking, this is, to my knowledge, the outline of the developmental cycle in all pathogenic entamoebae observed to this time. Important variations from Hartmann's description have been noted, particularly by Elmassian (1909), but as far as I am aware, the end stage in all types is a four-nucleated cyst, and this is the only end stage hitherto authentically described. It does not follow, however, because the end stage is a four-nucleated cyst that all four-nucleated cysts belong to the same species.

Subsequent to attacks of acute dysentery, when the stools are formed, when the characteristic symptoms have disappeared, and in cases in which there are no symptoms of acute dysentery, but rather those of intestinal disturbance, sometimes associated with constipation, and sometimes with diarrhoea, I have only once found organisms of Types A or B. In all other instances the entamoebae differed markedly from the large vegetative forms seen in acute dysentery, and were found under one of four conditions.

1. Following the acute dysentery in which Type B was observed, in the period when solid or semi-solid stools, without pus, blood and mucus, were being passed.

2. Following attacks of acute dysentery, but not of a severe form, when the intermediate type of entamoeba previously referred to as Type C was observed.

3. In cases in which there was a previous history of mild dysentery, or of intestinal disturbance. When admitted to hospital, however, such cases did not present symptoms of dysentery at any time.

4. In one case in which there was no history of dysentery at any time.

Although I followed four cases of Type A infection for a period of three weeks, and other cases for shorter intervals, I did not at any time observe in this type conversion to the latent types which I am about to describe. At times there were marked alterations in the nuclei and cytoplasm of the organisms, similar to those described by Hartmann (1912) for the pre-cystic stage of *tetragena* (figs. 5-9), and twice chromidia formation was observed, but although there was some improvement in symptoms, blood, pus, and mucus could always be demonstrated in the stools, and no cyst formation took place.

1. *Latent and encysted forms of Type B, Entamoeba tetragena.* In one case of this infection I observed the entire cycle twice, an

interval of three months intervening between the first and second observations. Each time the acute symptoms lasted for about five days, followed by a remission, with formed stools later. During the periods of observation no medicine other than small doses of salol was given, and this only once. With rest in bed the stools changed from dysenteric to watery, and in the latter was some bloody mucus.

In the watery stools decided changes in the entamoebae were apparent. The nucleus was no longer visible in most of the individuals. The size was greatly increased. The ectoplasm was less in quantity, and not well defined. Motility, however, was not impaired, and many of the organisms contained erythrocytes.

In stained specimens the organisms were more delicately alveolar. Many of the nuclei stained darker than normal, and distortion and irregularities of outline were frequent (figs. 5-9). In most of the organisms, however, the nuclei were typical of *tetragena*, although, as noted, in the fresh preparation they were ill defined.

This stage persisted for about a week, and then the stools became semi-solid. The organisms were still further reduced, averaging from 14 to 16μ (figs. 39, 41). In fresh preparations they were not easily detected, and frequently a high dry power, such as the Zeiss 8 mm. objective, had to be used to find them. They were very delicate, with scanty ectoplasm which closely resembled that of *Entamoeba coli*, although distinctly more refractive to one accustomed to observing the latter species. Visibility of the nucleus varied greatly, all degrees being observed. The cytoplasm was more markedly alveolar than at any other stage. A day or so later constipation set in, and several doses of salts were given to obtain specimens. The salts produced copious stools, in one of which a mass of bloody mucus was found. Examination of this demonstrated an enormous number of Type B vegetative organisms, in every way typical of *tetragena*. This is the only time I have found large vegetative forms coincidently with the type under discussion, although in other cases I tried by strong purging to get them.

In all subsequent stools from this case only the small forms were found. Many of these contained chromidia, which in fresh preparations showed as opaque bars and masses, and in stained as deep blue inclusions. Here the nuclei lost the typical *tetragena* characteristics, and appeared with only a small karyosome and a few grains of peripheral chromatin, and were very similar to the nuclei of Elmassian's *Entamoeba minuta* (figs. 26-31).

After dry fixation, and staining with Hastings and Giemsa, these little entamoebae presented a very characteristic appearance. The nucleus was a dark blue, with a small, deeply staining mass of erythrochromatin, generally arranged in a circle. The cytoplasm was granular, a dark blue with bands of lighter shading, and contained large amorphous masses staining in various shades of red. The chromidia stained deeply as heavy masses of blue. A comparison of figs. 127-129 with figs. 137-144 will show the difference between this phase of *tetragena* and *Entamoeba coli*.

Two days later there was a very great increase in the number of entamoebae, and numerous cysts appeared. In one 2 mm. field often several cysts and vegetative forms could be seen. In fresh preparations the motile entamoebae were the same as described above, and all degrees of nuclear visibility were observed. The cysts showed as highly refractive spheres, 10-14μ in diameter. These contained chromidia in opàque bars and crescent-shaped masses, often nearly filling the cysts. In some of the cysts delicate, refractive nuclei could be seen, varying in size. There were from one to four of these in each cyst. Sometimes single nuclei of large size, corresponding to what is also seen in stained specimens, were found, but no other stages of division were observed, although 'spindles' were common enough in stained specimens. As Darling (1913*d*) points out, cysts of the entamoebae can be distinguished from those of the flagellates by the crescentic margins of the latter. The flagellate cysts are also uniformly homogeneous, except at the margins (figs. 121, 122). In my opinion, the entamoebae cysts of any species are very typical in appearance, and once studied may be readily identified again if they occur in any number, as they nearly always do, at some time in the infection.

Wet fixed and stained specimens of this cyst-forming stage showed a great variety in nuclear and cytoplasmic appearances. As Hartmann (1912) notes, many of the vegetative organisms take on the typical nuclear appearance. And many degenerative forms were present, with associated variations in morphology. Chromidia of all shapes and sizes were present in both vegetative forms and cysts. In the degenerating organisms the cytoplasm stained faintly, and was granular. The nuclei exhibited all degrees of chromatin content and depth of staining, from an almost typical arrangement to a small karyosome without peripheral chromatin (figs. 35-40). In my experience, this variety in nuclear appearance is very characteristic of the cyst-forming generation of *tetragena*, and is one criterion of differentiation from *E. coli.*

Dry-fixed and Hastings and Giemsa stained specimens of this stage are equally characteristic, and I have not met similar appearances elsewhere. In proportion to their vitality the organisms stained from a dark blue in the healthy individuals to a very light colour in those far gone in degeneration (figs. 127-131). In the former the cytoplasm was granular, and in the latter it was almost homogeneous. The nuclei of the healthy organisms were dark blue, and set off from the cytoplasm by the heavier staining of their peripheries (figs. 127-129). The erythrochromatin was arranged in a compact circle. In proportion to the degree of degeneration the nucleus stained lighter, so that in organisms with very light cytoplasm it appeared as a colourless circle (figs. 130-131). In like proportion the erythrochromatin lost its ring shape and intense staining, and was broken up into fine threads and dots. The chromidia stained well, but did not appear in degenerating individuals. Their shape was the same as in wet-fixed or in fresh preparations (figs. 35-38, 128).

The cysts were typical. The cytoplasm was rather a light blue, obscured in the earlier stages by the deeply staining masses of chromidia. On account of the chromidia it is often impossible in the earlier stages to make out the nuclei; as the cyst matures, these appear as delicate rings of erythrochromatin. They vary in number, according to the age of the cyst. Dry fixation and Hastings and Giemsa staining does not show, except in one, two, three and four-nucleated stages (figs. 132-136), in my opinion, any of the phases of nuclear division so common in wet-fixed specimens, in which all stages of the development of the cyst may be followed without difficulty. In many of the adult cysts there are but few chromidia, and the four nuclei show very plainly.

Although in fresh preparations of the pre-cystic and cyst-forming generations the vegetative entamoebae are very similar to *Entamoeba coli*, a differentiation can be made as follows:—

1. *Entamoeba coli* in the vegetative stage is constant in morphology, varying only in phases of nuclear division. If the specimen is fresh, degenerating and atypical individuals are seldom seen. I have never observed what I myself would call true chromidia in vegetative *Entamoeba coli*, so that if these are present in vegetative individuals, the species is not *coli*.

2. Specimens of *coli* stained after any of the above methods show the characteristics of the species, as will be explained later. (See figs. 137-144).

3. Cysts of *coli* are, as a rule, larger than those of other species. When they are present in any number, the eight (and sometimes more) nuclei can be distinguished without difficulty. The cytoplasm is more refractive, is obscured more by food products that have not as yet been expelled than by chromidia, and is of a very striking pale grey colour.

4. The pre-cystic generation of *tetragena* will be followed shortly by encystment. This has been my experience in the case in which I followed the entire cycle twice, and in four other cases in which the entire cycle was followed. In two cases in which the pre-cystic generation was the first observed, encystment followed a few days later. The same process was observed by Darling (1913*d*) in the cases reported by him. As far as I know, the entire cycle, from the large vegetative forms associated with acute dysentery through the pre-cystic generation and through cyst formation has been observed only by Walker (1911), Darling (1912*c*), Craig (1913), and myself (1912). Walker notes also that the pre-cystic generation develops as the stools become normal, and is followed by encystment.

5. In four of my cases there was a double infection with *Entamoeba coli*, and entamoebae of the *tetragena* type, as demonstrated by the forming of eight- and four-nucleated cysts. In three of these the entamoebae and cysts were quite abundant, so that not infrequently both vegetative forms and cysts of each species could be seen in the same field, thus affording an excellent opportunity for comparative study. It was observed that in three

of the cases the four-nucleated cysts were always smaller than those of *coli*, that chromidia in the form of blocks and bars were very seldom present in the *coli* cysts, and that the vegetative forms showed constantly the type of nucleus shown in figs. 57-69 for *coli*, and in figs. 35-40 for the *tetragena* type. None of these cases had acute symptoms, so that the large trophozoites were absent in the *tetragena* type, but present in the *coli* type in three cases. In the fourth case all of the motile entamoebae were of the same size, and were unusually small, the vegetative forms not measuring over 12-14μ. Yet in this case, although the *coli* cysts were smaller than normal, those of the *tetragena* type were still smaller, and measured only from 7-10μ. Probably some circumstance of environment was responsible for this condition.

6. Since in cases in which the pre-cystic generation is present the symptoms are not urgent, one is justified in waiting a few days for the certain appearance of the cysts. If the dysentery has been latent for any period, the cysts will very likely be present at the time of examination. Personally, I have never seen an infection with *Entamoeba coli* in which its characteristic cysts could not be detected in a few days, and generally in such infections the cysts are present in nearly all specimens. Hence, by waiting two or three days, the diagnosis in these doubtful cases can be made positive by the detection of the typical cysts.

2. *The Cycle of the Intermediate Forms, Type C*

This type is related to Types A and B, as it shows in fresh preparations the characteristics of Type A, while in fixed and stained specimens it approximates in appearance to both types.

Although morphologically it stands between Types A and B, in its developmental cycle it shows marked peculiarities. Type C was found quite recently, in three cases, which I shall designate as Cases 1, 2, and 3, respectively. I have no record among my notes for the past 18 months of a similar organism showing the particular cycle found in the Type C entamoebae, but I have no doubt that Type C occurs here more frequently than my experience would indicate.

All three infections with Type C organisms were in European labourers, who were admitted to my section in a period of ten days.

Case 1 had been in the Canal Zone for two and one-half years; Case 2, for six years; and Case 3, for only two weeks. The last came here directly from France. None of the patients gave a history of dysentery prior to the attack for which he was admitted, although carefully questioned. Case 1 had had dysentery at intervals for two weeks prior to admission; Case 2, for two months; and Case 3, for eight days. In two of the cases the dysentery was complicated with malarial infection; in Case 1 tertian parasites were found, and in Case 2, æstivo-autumnal. In none of the cases was the dysentery severe at any time.

The morphology of the entamoebae was the same in all three cases, and, except for the apparent effect of the change in diet on the organisms, the course of the cycle was the same in each case.

On admission, the stools of Cases 1 and 3 were liquid, with bloody mucus and pus. The stools of Case 2 were formed, but coated with bloody mucus.

In fresh preparations the entamoebae were in every way identical with those of Type A, except for very occasional individuals in which the nuclei were easily discernible. In Case 1 the stools were watery for seven days, with some blood and mucus. In Case 2 they were partly formed and partly semi-liquid for six days, and in the bloody mucus of each stool entamoebae were found in varying numbers. In Case 3 the stools were watery for two days, with very little bloody mucus.

In Case 1 the morphology of the entamoebae was unaltered for the first five days, when the organisms lost much of their motility, and for the next two days showed very marked nuclear changes and ill-defined ectoplasm (figs. 6-9). In Cases 2 and 3 there was no alteration in the morphology until the change in diet. Ten grains of salol three times a day was given in Cases 1 and 2, and no medication in Case 3.

On admission, the patients were placed on a milk diet. On the eighth day in Case 1, the seventh day in Case 2, and the third day in Case 3, the diet was changed to include eggs, toast, soup, bread, and potatoes.

Coincident with the change in diet in each case the bloody mucus disappeared from the stools. In two days they were semi-solid in all three cases, although in Case 2 they had been formed previously.

Marked alterations in the morphology and activity of the entamoebae accompanied this change in the structure of the stools. These alterations will now be described in sequence, and, as noted, were the same in each case.

Although at the time of admission the entamoebae in fresh preparations were of the *histolytica* type, or Type A, in the wet fixed and stained preparations they were not true to type, as in other infections. For the most part the nucleus showed a condensation of chromatin under the membrane; a karyosome somewhat larger and not so compact as in Type A; and distinctly more chromatin distributed in small granules in the outer nucleus and under the membrane. A differentiation of centriole, clear zone, and surrounding karyosome, which is typical of Type B, could not be demonstrated with any certainty, nor could any definite cycle of the karyosome be made out, although traces of this were seen (figs. 17-21).

In Case 1 these nuclear characteristics persisted for five days, when marked alterations were observed. The entamoebae became smaller, and the nuclei were often deeply stained and distorted, while in some individuals there was apparent extrusion of the chromatin into the cytoplasm (figs. 6-9).

Chromidia formation now took place, and persisted for two days. Concurrently with the change of diet, the nuclei returned to their former appearance. In Cases 2 and 3 no nuclear alteration of importance was noted after the change in diet.

After the change in diet, in Cases 2 and 3 chromidia formation was also observed, and this phenomenon persisted in Case 1. Two days later cysts appeared in enormous numbers in Case 1, and in smaller quantity in Cases 2 and 3. I could not at any time demonstrate nuclei characteristic of Type B in the pre-cystic and cyst-forming generations in these three cases, although in cases of Type B infection from the start, the *tetragena* nucleus was very plainly evident in many of the organisms. There were, as in Type B infections, numerous degenerating organisms in the cyst-forming generation. The appearance of the entamoebae of Type C in the pre-cystic and cyst-forming generations is best judged from figs. 26-31, as compared with figs. 35-40 showing the same process in Type B.

Since I had these three cases under observation at about the same time, it may be thought that the peculiarities in structure of the entamoebae were due to some alteration in the reagents employed. I do not believe, however, that this is correct, for I had also under observation simultaneously with these cases another of a rapidly fatal infection. Specimens from this last, when fixed and stained with the same reagents employed in the other cases, showed typical Type A entamobae.

The interesting features of these three cases lie not so much in the morphology of the entamoebae as in the course of the cycle, especially in Case 3, in which there was a transition in three days from the large vegetative organisms associated with dysenteric symptoms to the cyst-forming generation. So short a period of transition has not as yet been reported. Another interesting point is that in Case 2, although formed stools were passed, the entamoebae were of the type associated with acute dysenteric symptoms. It will be remembered, however, that in this case the stools were coated with bloody mucus. Probably there were a few ulcers low down in the large bowel.

According to Hartmann (1912), and in my experience as well prior to these three cases, the cyst-forming generation is preceded at some time in the cycle by the appearance of vegetative organisms of the typical *tetragena* type. In these cases, however, I could not demonstrate any transition through this type, and the entamoebae appeared to pass directly from a modified *histolytica* type to cyst-formation. Apparently these three were primary cases, of relatively short duration, whereas in my cases in which the typical cycle of *tetragena* was observed, the infections were all of long standing, and the dysentery observed was very probably a relapse.

In two of these cases, Nos. 1 and 2, the change in diet seemingly brought about an alteration in the morphology of the entamoebae, and hastened the further development of the cycle. In Case 3, which was milder than the two others, the cycle might have developed without change in diet. I cannot state with any precision how far the change of diet was directly influential in determining cyst-formation, because I have observed other infections with the *histolytica* type, in which the diet was the same, fail to undergo any development of the cycle, although followed for three weeks.

Further observations on these interesting features of entamoebic infection are necessary to determine under what conditions the development of the cycle takes place, and these will be taken up when opportunity offers.

3. *Entamoebae in Cases with a History of Mild Dysentery, or of Intestinal Disturbance without Dysentery, while in Hospital.*

In four cases in which there was a history of previous more or less severe dysentery, but which presented on admission to hospital only symptoms of intestinal disturbance, the entamoebae found were of the type described by Hartmann for the pre-cystic and cyst-forming generations of *Entamoeba tetragena,* and were identical also with those found by me in following the cycle of the Type B infections.

In my total series, there were ten other cases, whose history demonstrated previous mild attacks of diarrhoea or of intestinal disturbance without marked dysenteric symptoms. All of these cases were admitted for diseases other than dysentery or diarrhoea, and six of them for malaria. The patients were European labourers, under my personal supervision. I may note here that the European labourer, especially the Spaniard or the Italian, nearly always associates dysentery with tenesmus and the passage of bloody mucus, and distinguishes very clearly between this clinical picture and that of diarrhoea. Whenever he has any intestinal disturbance, he notes the kind of stool that he passes, and I have found the history he gives with respect to this to be very accurate.

In these ten cases the entamoebae resembled very closely those described by Elmassian (1900) as *Entamoeba minuta.* There are biological as well as morphological differences between this organism as described by Elmassian, and certain phases of *tetragena* as described by Hartmann (1912), that should at least be made clear for diagnostic purposes. From my study of these cases I can confirm fully many of the points brought out by Elmassian as characteristic for *Entamoeba minuta,* although in one or two details, which will be noted, my findings differ somewhat from his.

In all cases, except one, clinical symptoms of vague abdominal distress, with alternating diarrhoea and constipation, were elicited, although on admission the patients had not complained of these. No dysenteric symptoms were present at any time after admission in any of the cases, nor did any of them give definite histories of severe dysentery. No pus, blood, nor mucus was found in the stools, even after repeated purgings and many examinations. In formed stools, for the most part, only cysts were found, but in the semi-solid and liquid stools after purging motile vegetative forms were observed, often in very great quantity. These entamoebae have very well-defined characteristics; they are not associated with acute dysentery; they are remarkably uniform in morphology, and degenerating forms are rarely encountered; and cysts are found at some time while the cases are under observation, for the most part in all specimens.

The morphology of the vegetative organism in the fresh preparation corresponds very closely to Elmassian's description. It is, as he notes, difficult to distinguish the entamoeba in the faecal material, and for this reason it may be easily overlooked, since it is not refractive, and does not contrast with the surrounding medium. Motion is sluggish. There is, in motion, a well-defined hyaline ectoplasm, which contrasts with the grey, alveolar endoplasm. Very few foreign bodies are seen in the endoplasm. The nucleus, according to Elmassian, is not visible. I could, however, observe it in some individuals as a delicate, refractive ring. The entamoebae are very uniform in size, measuring from 10-15μ, rarely above or below these figures. Chromidia are not found with any regularity, although not infrequently present. In the fresh preparation the cysts are very similar to those of the *tetragena* or to the intermediate (Type C) infections.

In wet-fixed and stained specimens the entamoebae show consistently a well-defined alveolar structure, especially after Schaudinn's and Zenker's fixatives. The pseudopodia are finely granular, as in other species. The nucleus maintains a very constant structure, and in this respect *minuta* is similar to *coli*, in that variations from type are seldom observed. It is spherical, except in division, and has a thick and regular membrane. The chromatin is in fine granules, collected mostly towards the periphery, and large blocks and segments under the nuclear membrane are not often observed.

The karyosome is seen as a small granule in the centre of the nucleus. Elmassian states that he did not observe any differentiation of this central chromatin into a karyosome and centriole, and in this respect my observations confirm his. In the pre-cystic and cyst-forming generations of *tetragena* such a differentiation is often very plainly seen, but certainly appears to be absent in entamoebae of the *minuta* type (figs. 26-31, 111-112).

Multiplication in the vegetative stage, according to Elmassian, takes place by two successive mitoses, so that a four-nucleated schizont is formed. I have seen many organisms which correspond to his description of this stage, but I cannot state with certainty if they are vegetative forms or cysts. Elmassian, however, pictures the four-nucleated stage with pseudopodia, which is to him conclusive evidence of schizogony. I found, in a specimen he sent to me, what appears to be the second mitosis. It has been demonstrated by several authors that *Entamoeba coli,* which forms eight-nucleated cysts, multiplies by simple division and by eight-nuclear schizogony as well, and if this be true I see no *a priori* reason why a four-nuclear schizogony should not take place in an entamoeba which forms four-nucleated cysts, especially when it is remembered that the total number of either *tetragena* or *minuta* infections hitherto reported as worked out by wet fixation and staining methods is not very large.

Cyst formation is initiated by the formation of chromidia; a change in the cytoplasm from alveolar to granular; and a swelling of the nucleus, sometimes, to twice its former diameter (figs. 42-45, 112). A spindle of considerable length is formed (fig. 46), after which the nucleus divides. Subsequently two nuclei, in all the phases from the resting stages to elongated spindles, are frequently observed (figs. 47-49). The end stage is a cyst with four small nuclei (figs. 52-53). I cannot state if autogamy occurs, although I have seen many of the phases of this process as described by Elmassian. The nuclei of the end stage, while superficially resembling those described for *tetragena* cysts, are more delicate, and show no karyosome formation (fig. 113), according to Elmassian. In all of my preparations of four-nucleated cysts, whatever the source, the nuclear structure was the same.

Unfortunately, at the time I studied these infections, my polychrome stains were not working well, and I cannot give an account of the appearance of these entamoebae after dry fixation and staining with Hastings and Giemsa. I believe, however, that the organisms, if stained by this method, would resemble closely those of the pre-cystic and cyst-forming generations of *tetragena*, and so could be differentiated from *Entamoeba coli*.

The entamoebae described as the 'latent' stage of *tetragena* infections, and those described above as *Entamoeba minuta*, are of importance; for just as Type A represents the common organism of acute amoebic dysentery in the Canal Zone, so these represent the organisms found after mild attacks of dysentery, or of intestinal disturbance. It is known that the 'latent' forms of *tetragena* infections can renew their vitality, and bring about a relapse, provided the possibility of auto-reinfection is excluded. I do not know if the organisms of the *minuta* type will take on a virulence that would result in acute dysentery, since in my cases they were quickly eliminated by the bismuth treatment described in the accompanying paper by Dr. W. E. Deeks. In Elmassian's case, however, the infection persisted for some years, and gave rise to considerable bodily and mental distress. Cats infected by Dr. Darling from one of these cases developed dysentery and died. The measure of pathogenicity of an entamoeba, however, can scarcely be determined accurately by the effect of artificial infection of cats, since these animals are notoriously susceptible, and, as I shall show in the next section, an infection which did not disturb the human host was fatal in a cat.

Whether *Entamoeba minuta* is a separate species, or whether it is a phase in the cycle of a pathogenic organism represented at times by Types A, B and C, will have to be determined by further research. As has been noted, Hartmann (1912) refers to *minuta* as identical with *Entamoeba coli*. In my experience, however, *Entamoeba minuta*, except perhaps when observed in the vegetative stage in fresh preparations, is entirely distinct in all of its phases from *Entamoeba coli*, and even in the fresh preparation it can be differentiated by careful study. Walker (1911), Darling (1913*d*) and Craig (1911, 1913) are of the opinion that *Entamoeba minuta* may be a phase in the cycle of *tetragena*. If so, it is a very well defined and singularly distinct phase, and its inclusion in the *tetragena* species will necessitate the giving up of several criteria now in use for phylogenetic classification. I must note here, however, that the entamoebae found in the cyst-forming generation, whether following acute dysentery or whether occurring as 'latent' organisms, were often morphologically identical, although the preceding generations showed wide morphological variation.

4. *Entamoebae not associated with dysentery*

One of my patients was a Spanish labourer, who was admitted for malaria. In the routine examination of his stool, Dr. Henry R. Carter, Jr., found a few entamoebae, and turned the patient over to me. The latter stated that he had been in the Canal Zone for about a year, and had never suffered from dysentery in his life. His stools were normal at the time of admission, and remained normal while he was in hospital.

After salts had been given, a semi-solid stool was examined. Two distinct types of vegetative entamoebae were found. In both the fresh and stained specimens one type corresponded exactly to the organism described in the preceding section, the *Entamoeba minuta* of Elmassian, and needs no further description.

The other type, of about the same size, was a sluggish organism, and possessed of marked peculiarities. Its pseudopodia, often multiple, sometimes attained a length equal to that of the entamoeba itself. They were slender, and very highly refractive. The endoplasm was markedly alveolar, almost vacuolated, and not refractive. Each individual, almost without exception, contained a highly refractive circular mass of what was evidently nuclear chromatin, in most instances surrounded by a clear zone of about 1 μ in thickness. Although a careful search was made, no evidence of a contractile vacuole could be demonstrated. There were also many non-motile granular, highly refractive bodies, each with a nucleus similar to that of the vegetative amoeba. These bodies were identical in appearance with the well-known cysts of the so-called free living amoeba of the *limax* group. They were easily distinguished from the cysts of the associated entamoebae by their single, shining nucleus, their granular cytoplasm, and the absence of chromidia.

Also after wet fixation and staining these organisms were identical in appearance with those of the *limax* group. The nucleus in most instances consisted of a large, circular, deeply-staining karyosome, outside of which was a narrow, unstained space, with an outer margin that stained very faintly (figs. 114-115, 117-118).

Some of the nuclei of the vegetative forms were elongated (fig. 120), others dumb-bell shaped, while some individuals had well-defined, double nuclei. These amoebae were the same morphologically as those described by Chatton and Lalung-Bonnaire (1912), who found their specimens in the stools of a patient from Indo-China, who had suffered for many years with a 'diarrhée chronique intermittente.' This comparison I could verify, from a slide very kindly sent to me by Dr. Chatton. He and Lalung-Bonnaire classified their amoebae as *Amoeba limax* (*Vahlkampfia punctata*, n. sp.). They appear also to be identical with specimens from several sources in my possession, which were cultivated from human stools.

The cysts were small, rounded bodies, in which the nuclei were identical with those of the vegetative forms, but whose cytoplasm was more granular, with a well-defined single or double membrane (figs. 116, 119). They were also identical with the organisms in a preparation sent by Dr. C. M. Wenyon, from the case described by him (1912).

Trichomonas intestinalis was present also in these specimens, both vegetative and encysted forms (figs. 150, 121-122).

A cat infected *per os* by Dr. Darling, after a somewhat longer incubation period than usual, developed dysentery, and died. The stools contained many entamoebae of the type described by Darling (1913*d*), very many *Trichomonas*, but no amoebae of the *limax* type. Neither Dr. Darling nor I were successful in culturing these amoebae.

The stools of this patient were examined alomst daily for a space of three weeks. In this time there was no change in the morphology of either of the two types of organisms described. Sometimes one type would be more plentiful than the other, and there was a daily variation in the number of cysts of either type. During the period of observation the *minuta* forms preserved their remarkable uniformity of appearance, varying only in the phases of nuclear division, and remaining true to type in these. Except for like variations the *limax* forms were also constant in appearance.

In order to classify the species present in this infection, I submitted slides to well-known authorities in Europe and in the United States. All agreed that the organisms of the *minuta* type were not *Entamoeba coli,* most of them stating that they were *Entamoeba tetragena,* on account of the four-nucleated cysts. There was, however, a diversity of opinion as to the classification of the organisms of the *limax* type. Three held that these were a true free living species, parasitic for the time being in the human intestinal tract, provided that re-infection during the period of observation were excluded. One stated that the '*minuta*' forms were those of the latent stage of *tetragena* and the *limax* forms were degenerating phases of these, as described by Hartmann (1912), especially in that author's fig. 31. Another authority was of the opinion that the *limax* forms were the amoeboid stage of *T. intestinalis,* although he did not give an opinion as to the cysts.

Although the *minuta* forms may eventually be classed as a phase of *Entamoeba tetragena,* I do not believe that the *limax* forms were degenerating organisms. For one reason, these *limax* forms were not found in any other of my *tetragena* or *minuta* type infections. For another, degenerating forms of *tetragena,* when present in any quantity, show a very great variety in nuclear and cytoplasmic structure, while in my specimens these *limax* forms were, as noted, very constant in morphology.

T. intestinalis was present in these specimens, but its cysts are, in my opinion, quite different from those described for *limax* amoebae. *Trichomonas* infection is very common here, and the only cyst-like bodies that I have seen in single infections with this parasite were those of the form shown in figs. 121, 122. There is, I am aware, some difference of opinion as to whether the organism shown in figs. 121-122 is a *Trichomonas* cyst. According to Chatton and Lalung-Bonnaire, Alexeieff classes it as a yeast, *Blastocystis enterocola.* But these organisms are always associated at some time, in my experience, with *Trichomonas* infections, and I do not understand why the cat referred to above was infected with *Trichomonas,* if not from them.

The possibility of auto-infection, or infection from external sources after admission was carefully considered, and I believe eliminated. The patient was on the same diet and drinking water supply as the other patients, and examination of control stools from these failed to reveal any amoebae. In some of the specimens the *limax* forms were two to three to every oil-immersion field, and there would have to be ingested an incredible number of cysts daily to produce such a supply of vegetative forms, if this quantity is to be accounted for by the development without multiplication of the cysts. Moreover, the presence of many organisms in various stages of division demonstrated clearly enough that the amoebae were multiplying in the intestine of their host.

From this evidence I believe this was a true infection with a species of so-called free living amoebae, which for the time being assumed the rôle of facultative parasites or commensals. The case, then, presents two important points: (*a*) the presence of a pathogenic species without a history of dysentery; and (*b*) the parasitic existence in a human host of what is commonly classed as a free living species.

The first point demonstrates again the necessity for the careful examination of stools in patients in the Tropics, or who have returned from there. This patient was a 'carrier' of pathogenic organisms, which, if not parasitic for him, certainly would have been for others, in a stage entirely suitable for the transmission of infection. It is also possible, whether the entamoebae be classed as *tetragena* or *minuta*, that the infection might later have taken on virulence, with the consequent development of dysentery, or possibly of a liver abscess.

The importance of the second point is well brought out by Chatton and Lalung-Bonnaire (1912) in their interesting contribution. In their case they did not find the amoebae in the fresh specimen, but only after the examination of fixed and stained specimens. Their cultures on Musgrave and Clegg's medium were not positive until the eighth day. The cultures made from my case at my request, by Dr. Darling, were not continued after the fifth day, and my own failure very probably may be attributed to inexperience in this work, and the neglect to sow the medium with a proper symbiotic bacterium.

Chatton and Lalung-Bonnaire are of the opinion that although free living amoebae have repeatedly been cultured from the stools of dysenteric and non-dysenteric patients, up to the time of their communication no report had been made of facultative parasitism of these in the human intestine. Such free living amoebae as had been cultivated from human stools were thought to have originated from cysts taken in with food and drinking water, since until their observations direct observation had failed to show amoebae of the *limax* type in the stools of warm-blooded vertebrates, and especially man. If this be true, then the authors are the first clearly to demonstrate a free living species, facultatively parasitic in man. Walker (1911) did not demonstrate any of the amoebae that he cultured in specimens from stools, but Gauducheau (1909) found in human stools amoebae similar to those which cultivated, and called them *Entamoeba phagocytoides*. These organisms were also very probably found by Lesage (1905) and Noc (1909) in human stools. However, I leave the question of priority in this instance to those who are more familiar than myself with the work of these authors.

Chatton and Lalung-Bonnaire state that this facultative parasitism of a free living species, and its multiplication in the human intestine, will explain not alone the cultures obtained from human stools, but will make clear also why amoebae have been cultivated from liver abscesses. 'It is possible,' they say, 'to suppose that these amoebae follow the way opened from the intestine to the liver by the "perforating entamoebae," a way seeded with bacteria from which they take their nourishment.' This point of view, to those who do not admit a relationship between the free living and the parasitic species, offers an explanation of why cultures may be sometimes obtained from liver abscesses, without depreciating the technique of experienced bacteriologists.

The case reported above by me is, I believe, the first in which parasitic entamoebae and free living amoebae have been found in any number in the same individual, the organisms of each species undergoing simultaneously a cycle from vegetative forms to cyst formation.

Wenyon (1912) gives a very interesting account of an infection with a free living species, in a native of India who was admitted to a London Hospital. In this case, a small amoeba, in size from 3-10μ, was found in enormous numbers in the stools, sometimes as many as 20 to the field of a Zeiss 1/12 objective. There were no symptoms of dysentery. An attempt to infect a cat failed, as in my case, although there was no trouble in infecting cats with the pathogenic species from other individuals. Wenyon does not state if cysts were also present, or if he made cultures.

[A comparison of my preparations with a specimen from this case kindly lent to me by Dr. Wenyon, shows that the organisms from each patient were morphologically identical. This would seem to demonstrate that these organisms were not degenerating *tetragena*, because Dr. Wenyon failed to infect his cats, and the slide he sent shows no recognized forms of the pathogenic species. Also Drs. Chatton and Lalung-Bonnaire were successful in culturing an amoeba morphologically identical with the organism in my patient, which, in the light of facts at present accepted, would not have happened had the organism in question been a *Trichomonas* cyst, or if it were a degenerating *tetragena.*]

ENTAMOEBA COLI

Although the earlier writers, Grassi (1879), Calandruccio (1889-1890), and especially Casagrandi and Barbagallo (1895) described with considerable detail the morphology and also to a great degree the life cycle of the non-pathogenic entamoebae, and though Councilman and Lafleur (1891), and Quinke and Roos (1893), had noted the absence of dysenteric symptoms in many instances when entamoebae were present in the faeces, it appears that Schaudinn was the first to apply to the study of *E. coli* modern methods of fixation and staining. Prior to Schaudinn's communication several species of non-pathogenic entamoebae had been described, Celli and Fiocca (1895) enumerating no less than six such found in man, Quinke and Roos one, and Kruse and Pasquale (1894) two. More recently Koidzumi (1909) as *E. nipponica*, Lesage (1908) as *E. tropicalis*, and v. Prowazek as *E. williamsi* (1911), *E. poleki* (1912*a*), *E. hartmanni* (1912*b*), and *E. bütschlii* (1912*b*), have described as separate species entamoebae that apparently are various phases in the cycle of *E. coli*. (*E. nipponica* is probably described also from degenerating phases of *E. histolytica*.)

The literature pertaining to *E. coli* is relatively scanty when compared with that of the pathogenic entamoebae. However, excellent accounts of the life history of this organism are presented by Brown (1910) and by Craig (1911) in their monographs, while Werner (1911) has devoted especial attention to the species. The following brief historical note is abstracted from his chapter on *Entamoeba coli*, in v. Prowazek's 'Handbuch der Pathogenen Protozoen.'

Grassi was one of the earliest, if not the earliest writer to describe the organism now known as *E. coli*. He first observed the entamoeba in 1879, and noted that it was non-pathogenic. Working partly alone, and partly with Calandruccio, he found and interpreted correctly the cysts, and described the process of cyst formation. Calandruccio confirmed Grassi's work, and infected himself *per os* with the cysts, which was, as Werner notes, the first time that such an experiment had been carried out. Later Quinke and Roos, and Schuberg (1893) described organisms very closely akin to *E. coli*.

To Grassi's pupils, Casagrandi and Barbagallo, belongs the credit for giving, in 1895 and 1897, for the first time a thorough description of the vegetative forms, and also of the hitherto imperfectly described developmental cycle within the cysts. Schaudinn, however, was the first writer clearly to differentiate the species of entamoebae found in the human intestinal tract. His account of *E. coli*, in fresh as well as in fixed and stained preparations, and in the vegetative and encysting stages, is in all essentials morphologically correct. But later research has shown, as it has shown also in his account of the biology of *E. histolytica*, that there are strong reasons to believe he misinterpreted various important occurrences in the cycle, especially in his descriptions of schizogony and of autogamy.

Since Schaudinn's communication *E. coli* has been described by several writers, but as far as I can ascertain, the only other accurate accounts accompanied by plates showing properly the appearance of the organism after wet fixation, are those of Werner (1911), and of Hartmann and Whitmore (1912). Werner accepts Schaudinn's description and interpretation of the phases found in encystment, except for minor variations, and he gives two pictures which he interprets as the result of eight-nucleated schizogony according to Schaudinn. But Hartmann and Whitmore, while acknowledging the morphological correctness of Schaudinn's observations, differ entirely with him, as will be pointed out, with respect to the mechanism of the methods of schizogony, simple division, and nuclear division within the cysts.

Mention must be made here of the work of Wenyon (1907). His description of the entamoeba found in the intestines of mice, *Entamoeba muris*, is almost identical with Schaudinn's of *E. coli*, as he notes. Wenyon had the advantage of studying *E. muris* in preparations from the intestinal canal, and so could give many interesting details as to its habitat and methods of living which have not been recorded for *E. coli*, since the latter organism has been studied only in the faeces of men and of cats. It is my intention at an early date to try to infect mice with cysts of *E. coli*, and if the experiments should be successful, to compare the organisms thus found with *E. muris*. In this way it may be possible to complete the cycle of *E. coli*, in the manner in which Mercier (1909) completed that of *E. blattae*.

My own material for the study of *Entamoebae coli* consisted of fresh and stained preparations from fourteen infections with this organism. In four of these infections *E. histolytica vel tetragena* was present also, two were in patients, one of whom, a child, had a mucous colitis, and the other, diarrhoea due to chronic nephritis. Of the rest, three were in patients who have been in Ancon Hospital for several years. One of these latter infections, from which most of the preparations for my drawings were taken, is of particular interest. The infection was in a senile negro patient, suffering from a chronic cardio-renal condition, who had been for six years in Ancon Hospital, and the infection is known to have been present since admission. From the stools of this patient I have obtained at various times all the types and variations seen by me in other infections, and it has been possible, I believe, logically to construct a cycle for *E. coli* similar to that presented in Part III for *E. histolytica*.

DISTRIBUTION

Although most writers agree that *E. coli* is a frequent inhabitant of the intestinal tract of tropical natives, and is also widely distributed throughout the North Temperate Zone, at present the organism is not found to any extent among the working force of the Isthmian Canal Commission. Formerly it was not infrequently encountered, and not seldom mistaken for the pathogenic species (James, 1912*a*). A routine examination is made of the stools of most patients admitted to the medical side of Ancon Hospital, and though by far the greater part of the specimens so procured follow the administration of saline cathartics, *E. coli* is not often demonstrated. Very probably a more careful search would increase the percentage of positive findings, but my personal experience in searching for infections indicates that this entamoeba is not at all common among our West Indian negro labourers at this time, and is very infrequent indeed among the European workers, most of whom are Spaniards, and the American employees.

The native population, however, is much more heavily infected with *E. coli*, as it is also with other intestinal organisms, and I find it not infrequently in the stools of Columbians who come to this hospital for treatment from Cartegena and other places on the Northern coast of Columbia.

Since my study for the most part confirms the work of Werner and of Hartmann and Whitmore, I shall follow in my account the logical order in which these authors present their views.

SIZE

Entamoeba coli, and the closely-related organisms described by the above-mentioned authorities as separate species, varies greatly in size. The pathogenic types for the most part appear in the stools as individuals in the same phase of the life cycle, and in a given preparation show no great disparity in their dimensions except that which can be accounted for as following simple division and the intermediate stages subsequent to this as the newly-divided organisms increase in size. *E. coli*, however, in many instances in the same preparation, presents a remarkable diversity in mensuration, ranging from small organisms not much larger than the nucleus itself (figs. 96-97) to very large entamoebae which at rest measure from 40-60μ in diameter (figs. 57, 63, 138, 139), and in extension are even longer (figs. 62, 69, 152, 153). In general, it may be stated that when the faeces are liquid or semi-liquid, as in acquired or artificially provoked diarrhoeas, the entamoebae are largest, and become smaller as the faeces become formed. There is, as will be shown later, a well-marked cyclical variation in size, similar to that encountered in the pathogenic species, which in favourable circumstances may be well demonstrated, and in many respects this variation is *en rapport* with particular phases in the life cycle.

Here I may note that the term 'young,' which one finds frequently applied in the literature to the small entamoebae, is often a misnomer, for at the end of any given period of vitality the organisms, whether of the pathogenic or non-pathogenic species, are in general the smallest, relatively and actually, observed during the stages of cyclical activity.

There is in *E. coli*, as in the pathogenic species also, a 'latent' period, during which the organisms, as they appear in the faeces, are relatively small and exhibit a uniformity of size, structure, and nuclear detail, varying only in the phases of cyst formation (figs. 58, 60). I have observed such a latent period for three weeks in the infection particularly referred to above. But when the entamoebae were stimulated by the effects of strong purging, not only did very large vegetative forms appear after a few days in the liquid stools, but they persisted until the stools were formed again (figs. 66, 69, 152, 153), although at this time many smaller pre-cystic organisms were also found (figs. 59, 64, 70, 76).

With respect to the habits of *E. muris*, Wenyon (1907) states:—

'These amoebae live in greatest numbers in the caecum. They occur to a lesser extent in the upper parts of the large intestine, and are never found above the caecum. In the ordinary course of events the amoebae do not escape from the body of the mouse, but, in diarrhoea, free forms may be found in the faeces. In normal faeces only encysted forms occur.

'In the caecum the amoebae live free amongst the caecal contents and also upon the epithelial surface. They may even enter the glands and make their way to the remotest extensions of these. There is never any indication of their being able to penetrate the epithelium. The amoebae live in the company of

Trichomonas, *Hexamitus*, numerous bacteria, yeast cells and spirochaetes.'

I believe that the same is true of *E. coli*, since in formed stools only cysts are found. But the large vegetative organisms do not always come down with the first liquid stools after saline cathartics; frequently they are not found until some days after the purge has been exhibited. From this one may infer that following artificial stimulation there is a period of active increase in numbers, during which the entamoebae increase in size as well as in quantity high in the bowel, perhaps in the caecum. Frequently after the induction of artificial diarrhoea I have found for the first few days only a few organisms in the stools, and later a very large number, sometimes two or three to every low power field.

These facts will account for the diversity of measurement given by various authorities. Celli and Fiocca (1894), in their description of the six non-pathogenic species, gave the diameters as between 5 and 10 μ. The fact that none of their measurements approaches that of a normal *coli* cyst (15-20 μ) makes it very probable that they had to do either with an atypical *coli* infection or perhaps with another protozoon. For, as Craig (1911) notes, a description of an entamoeba measuring only 5 μ, or less than the diameter of an erythrocyte, may well be viewed with suspicion. Werner (1911), although he states that the size of *E. coli* varies between wide limits, gives pictures of forms as small as 5 μ, and of these his fig. 4 might also be interpreted as a free nucleus, or as such an organism as is shown in my figs. 63 and 152, within the entamoeba, while his figs. 6 and 7 might represent phases of flagellates, as Hartmann and Whitmore note. Werner's pictures of these little entamoebae, if they are such, were drawn from life, but in fixed and stained specimens phases of flagellates very similar to them are found (see fig. 150).

Casagrandi and Barbagallo (1897) give the dimensions as between 10 and 50 μ. Brown's (1910) measurements are from 12 to 25 μ, and both he and the former state that organisms exceeding the latter measurements are rarely found. Prowazek gives the diameters of *E. williamsi* as 5-25 μ; *E. hartmanni* as 4-13 μ; *E. bütschlii* as 10-24 μ; and *E. poleki* as 10-12 μ. Doflein's (1911) measurements are 7-70 μ.

Hartmann and Whitmore state that the average diameter of their vegetative forms was 20-40μ, and the smallest observed by them was was 10 μ.

My measurements show a very wide variation. In one case, observed over a period of two weeks, all the vegetative forms were from 8-15 μ, while the eight-nucleated cysts averaged only about 10 μ, some being even smaller. Unfortunately, stimulation by purgation was not tried. In another, the vegetative forms were all between 20 and 25μ, while the cysts were about 15 μ. In this case I could obtain specimens from a very few stools only, and did not have the opportunity to observe the infection for any time. In all my other cases there was a great disparity in dimension, the entamoebae measuring from 10 to 50 μ, and often very much more in extension, while sometimes all gradations between these limits could be observed in the same preparation.

The above data demonstrate very plainly, as Casagrandi and Barbagallo noted (quoted by Werner), that size is of no specific value in the determination of *E. coli*, if one uses as a criterion of the species the formation of eight-nucleated cysts. A marked variation of size in many of the entamoebae in the same specimen, however, points to *E. coli*, since, as noted, the pathogenic species tend rather to a uniformity of size at any given point in the cycle, and the very large vegetative forms are rarely found associated with cyst formation, and then only at the beginning of a relapse or after strong purging, as I found in one case (James, 1912*b*).

CYTOPLASM AND MOTION

The cytoplasm and the motility of *E. coli* vary greatly, and as a rule in proportion to the phases of the cycle. In general, it may be stated that in the large organisms found in diarrhoeal and liquid stools, the cytoplasm is clearer, less granular and more alveolar and vacuolated (figs. 57, 62, 69, 137-139), and the movement relatively more active; while in the semi-liquid and denser stools the cytoplasm is less refractive, the alveoli and vacuoles are fewer and smaller, the granules more plentiful and larger (figs. 59, 61, 71, 140-142), except in the cysts, and the movement less active. Between the extremes are found all degrees of variation, sometimes even in pasty faeces. It is small wonder, then, that authorities differ so pointedly as to structure and motility, since these are so variable. And yet, if these characteristics of *E. coli* are taken in their sum, and not individually, they serve as definite factors in distinguishing between this organism and the pathogenic species, as far as morphology is of value in such a distinction. The structure of the cytoplasm, in wet-fixed preparations, is best seen after stains such as Mann's, Lichtgrün, and Carmine, which demonstrate the details better than do Eosin, Acid-Fuchsin and Iron Hematoxylin.

As in other entamoebae, the cytoplasm is differentiated into ectoplasm and endoplasm. In fresh preparations the ectoplasm is best demonstrated in the large, actively motile organisms, and in those motionless forms often seen in semi-liquid stools, in which the endoplasm is contracted into a circular mass in the centre of the entamoeba. In the vegetative organism at rest the ectoplasm is not as a rule visible, but in the pre-cysting forms to which I have just referred it is often plainly seen as a broad, circular sheet. Viewed under favourable conditions, it appears as an almost homogeneous structure, whose very delicate granules can be made out only with a high power and suitable light. In vegetative organisms in motion the ectoplasm is not difficult to demonstrate, as some writers state, and can easily be seen with a good lens and proper illumination. Its refraction is less than that of the more granular endoplasm, and this difference is owing to the chromatic qualities of the granules of the latter, which are quite refractive, for in many organisms the difference is one rather of homogeneity than of a true refraction of the entire ectoplasm. According to Werner (1907) there is a very fine line of demarcation between the endo- and ectoplasm that gives the impression of a 'Brüchpforte' which must be broken through by the forming pseudopodia. In motile organisms this appearance is difficult to demonstrate, and is seen only in the brief interval between the formation of the ectoplasmic pseudopôd and the flowing of the endoplasm into it. This line of demarcation is very well seen, however, in those resting organisms previously referred to, in which the ectoplasm is so plainly visible.

In the vegetative organisms associated with acute dysentery, it is typical of the pathogenic species that the ectoplasm is actually and relatively more refractive than that of *E. coli*, while the line of demarcation between it and the endoplasm is easier to demonstrate. But the ectoplasm of *E. coli* has the same degree of refraction in all stages of the cycle, and does not vary in this respect, as does that of the pathogenic species.

In wet-fixed specimens of the vegetative organisms, according to the stain used, the ectoplasm appears in most instances as a pseudopod or pseudopodia of very finely granular structure. Indeed, the granules are so fine as to give the appearance of a delicate fibrillar network, with relatively open spaces. In dry-fixed specimens, stained with Hastings and Giemsa, it is more often and more abundantly demonstrated, appearing as an almost homogeneous sheet of a light blue staining substance (figs. 137, 139). The deeper staining endoplasm is often collected at the margin of the entamoebae in heavy granules (figs. 140, 141, 144), and should not be mistaken for ectoplasm. After staining with iron-hematoxylin, there is little difference in the appearance of the ectoplasm in the pathogenic and non-pathogenic entamoebae (figs. 12, 14 and 59). But in dry-fixed and Hastings and Giemsa stained preparations the difference is marked, and very constant, as first noted by Craig (1905). Compare figs. 125-126 with 137, 139.

As Werner notes, the endoplasm is distinguished from the ectoplasm by its granular structure. The endoplasm of *E. coli* is made up of a semi-fluid ground substance, in which are numerous granulations that vary greatly in size and arrangement. Werner states that Casagrandi and Barbagallo differentiate two kinds of granules; one the 'elementary granules,' and the other the 'excretion granules.' The former are very small, of approximately similar size, while the latter are large, polymorphous, and darker in colour. These granules correspond to those described by Minchin (1912); the elementary granules to the 'microsomes,' and the excretion granules to the 'metaplastic bodies,' which Minchin states are 'by products of the upward or downward metabolism of the organism.' In the large, active vegetative forms and in the smaller organisms resulting from simple division of these, as well as in any of the trophozoites when the vitality of the cycle is waxing, the smaller granules predominate, and the structure of the cytoplasm is more alveolar and takes a lighter stain (figs. 62, 66, 67, 69, 151-153), while in the pre-cystic and cyst-forming generations the larger granules are in excess, the entire endoplasm is more granular and less alveolar, and the body of the organism is of a darker colour (figs. 58-60, 64, 70, 72). In this respect it is significant that the more alveolar and vacuolated organisms are actively phagocytic and frequently stuffed with inclusions which they possibly may use for food (figs. 63, 152), while the denser entamoebae show but few inclusions.

However, in so far as fixing and staining are criteria, there is apparently no chemical difference between the large and the small granules, except that the former stain more deeply, and this is true even after polychrome dyes.

These facts regarding the cytoplasmic granules are true also of the pathogenic entamoebae, but in these the structure of the endoplasm at stages of the cycle corresponding to similar stages in *E. coli* is more alveolar, and at times in large organisms of the *histolytica* or the *tetragena* type it approaches an areolar formation.

The spaces between the granules in the endoplasm of *E. coli* vary greatly in size, and, as Werner states, in the living organisms as well as in fixed and stained specimens, these spaces show all degrees of gradation, from small spots to large vacuoles, and this in the same entamoeba (fig. 153). It is not easy to distinguish between inter-granular spaces and the small vacuoles, but as the latter become larger they show, when in proper focus, a definite circular shape with a well-defined margin, while the alveolar spaces are often irregular in contour. This structure is best shown after wet fixation (figs. 57, 68, 69, 152). Several adjacent vacuoles not simultaneously in focus will present an irregular outline (fig. 66), or the margins may be indistinct (fig. 60).

In the living entamoeba Werner describes vacuoles of two classes. In the first the vacuoles contain only gas (gasgefüllten), and these constitute the majority. When the entamoebae are in active motion these vanish as quickly as they are formed. Often they join together and again separate and disappear. In the second class are the vacuoles which contain food or phagocyted inclusions. Such vacuoles retain their form during movement, and are constant. These vacuoles vary greatly in size, some being larger than the nucleus (figs. 63, 152). From my own study I can confirm these observations of Werner's, and I would add that sometimes the endoplasm is stuffed with vacuoles containing inclusions (figs. 63, 152).

It is a question how far the foreign bodies observed in the vacuoles serve as food, how far they are truly parasitic, and how far they are merely phagocyted. Some appear to be commensals. *Entamoeba coli* is a very voracious organism, and is cosmopolitan in its taste. With the exception of erythrocytes, every body cell and degeneration form of these found in the intestine, every organism, bacterium, yeast, crystal, and vegetable cell seen in the stool, may at some time be observed phagocyted. In his description of the closely-allied *E. muris*, Wenyon mentions having seen a very large vacuole full of *Trichomonas*, all swimming actively. I have seen one motile *Trichomonas* in a small vacuole, and also I have frequently found motile bacteria in the vacuoles. Fig. 137 shows two *Trichomonas* and a filamentous organism phagocyted by the entamoeba.

Notwithstanding its voracity, *E. coli* at times appears to be selective in its food. In one of my preparations, with the exception of a few bacteria the only organisms phagocyted belonged to what I took for a species of yeast, although they may be some species of minute protozoa, and this in spite of the presence of very many *Trichomonas* and a great variety of the normal intestinal flora. Most of the entamoebae contained these little cells, sometimes in great number (figs. 63, 152).

Whatever *E. coli* takes up is promptly enclosed in a vacuole, and such a vacuole often unites with another or more to form larger vacuoles. When organisms of different kinds are phagocyted, it is not unusual to find different species in the same vacuole (fig. 63, bottom vacuole).

I am unable to state to what extent digestion takes place within the vacuoles, if indeed it takes place at all. I have observed living *E. coli* for several hours, in fact, until they died, without noting any change in the phagocyted inclusions. However, conditions of life under the microscope, especially those due to the effect of light on entamoebae whose normal habitat is in the lightless intestinal lumen, may very well not be normal. In wet-fixed preparations alterations may be noted in the staining qualities of the inclusions, and this is especially true of those shown in figs. 63 and 152, yet the same alterations were noted in identical bodies free in the intestinal contents. On the other hand, the selection of particular organisms at certain times would point to some definite purpose, as is true of the selection of erythrocytes during the period of ulceration by the large vegetative forms of the pathogenic species.

I am also unable to state if true parasitism of *E. coli* with other organisms occurs. I did not see anything in my own preparations which I could positively interpret as this, although some of the entamoebae contained a proportion of inclusions greater in volume than that of the cytoplasm itself. Wenyon (1907) believes that true parasitism of *E. muris* can be demonstrated, and Elmassian (1909) describes an organism that is either a parasite or a commensal that went through reproductive phases in *E. minuta*. True parasitism of both nucleus and cytoplasm in the free living species has been described by Dangeard (1894), and by Chatton and Brodsky (1909).

The importance of vacuoles in determining the identity of *E. coli* has been variously interpreted. Probably the personal equation in stating definitely the difference between a large alveolus and a vacuole has not a little to do with the present disparity of opinion. Brown (1910) states that vacuoles are of common occurrence in *E. coli*, while Craig (1911) gives as his opinion: 'The absence of vacuoles in this species is in striking contrast to the numerous vacuoles observed in *E. histolytica* and in the other pathogenic entamoebae, and is of some value in the differentiation of species.' Doflein (1911) makes no mention of vacuoles. Werner (1911), and Hartmann and Whitmore (1912) attach much importance to the vacuolated appearance of *E. coli*, and the latter are of the opinion that vacuoles containing inclusions other than erythrocytes (food-containing vacuoles) serve well for a point of differentiation between *E. coli* and other species.

With this latter opinion I agree. In a former communication (1912*a*) I stated that true vacuoles were met with more constantly in *histolytica* than in *coli*, basing my opinion on the results of a differential count with fresh preparations. Later study has shown me that I took the large alveoli in those *histolytica* which did not contain erythrocytes for true vacuoles, and also that I was working at the time with a pre-cystic cycle of *E. coli*. I made the very common error of generalising from too scanty material, and not taking into account the entire life cycle. It is certain, however, that when erythrocytes are phagocyted in any number by the pathogenic species the number of vacuoles is in proportion to the extent of such phagocytosis, while in *E. coli* small and large vacuoles are common, without regard to phagocytosis, in the large vegetative forms.

Except in the stages of encystment, to be described later, the formation of vacuoles is to a marked degree a function of the large vegetative entamoebae. In all species of the entamoebae of man, the presence of such forms denotes the period of greatest activity in the cycle, and undoubtedly the vacuoles have to do with the active nutritive processes at this time. In *E. coli* both ephemeral as well as relatively permanent vacuoles are most plentiful in this stage, while in the pathogenic species the vacuoles are not so abundant, unless, as noted, there is an active phagocytosis of erythrocytes. Pathogenic entamoebae not containing erythrocytes will for the most part show an alveolar structure, with relatively large spaces and few vacuoles (figs. 17-21, 22-25*b*). Only occasionally will there be included bacteria or stool detritus at this stage, and, in my experience, never flagellates. As the cycle ages, the vacuoles are fewer, until the cyst-forming generation is reached, when they reappear.

On the other hand, the active vegetative forms of *E. coli* are truly characterized by the presence of numerous vacuoles, ephemeral (without inclusions) and constant. An entamoeba containing even a small number of vacuoles with inclusions other than erythrocytes, and a varying number of vacuoles without inclusions (figs. 57, 62, 67, 69), or many vacuoles with inclusions is a *coli*, just as an entamoeba filled with erythrocytes belongs to the pathogenic species.

E. coli occasionally phagocytes erythrocytes, when these are present in any number. It is, however, the consensus of opinion that it never phagocytes more than a few at a time. Experimentally, Craig could not demonstrate phagocytosis of erythrocytes by this species, nor could I, in many experiments, although Craig states he had no difficulty in demonstrating such experimental phagocytosis by the pathogenic species. Gauducheau

(1909) states that culture amoebae freely phagocyte erythrocytes. Werner states: 'Since *E. coli* lives in healthy intestinal tracts, included erythrocytes are not observed.' This statement is only partially correct, because *E. coli* not infrequently is found under diarrhoeal and dysenteric conditions. I have observed four cases of double infection with *coli* and *histolytica*, in which erythrocytes free in the stools were plentiful, and did not witness phagocytosis to any extent by *coli*, although *histolytica* phagocyted the erythrocytes freely.

If the fact that under any conditions *E. coli* only seldom phagocytes erythrocytes can be substantiated completely, as by further observations on mixed infections with the pathogenic species, or on infections with *E. coli* and the Shiga bacillus, a point of much importance in the differentiation of species will thereby be established. Personally, I am fully convinced that entamoebae containing regularly few or many erythrocytes are always of the pathogenic species, and are never *coli*. It may appear singular that so omnivorous an organism as *E. coli* should refuse erythrocytes as it does, but it is equally as singular that the pathogenic species refuse practically to the same extent to phaogcyte anything but erythrocytes, and occasional bacteria.

THE NUCLEUS

The following description refers to the nucleus of the vegetative stages. The nucleus during encystment will be described later.

The nucleus of *Entamoeba coli* is granular in type. Minchin (1912) distinguishes two types of nuclei in the Protozoa.

'The structure and appearance of nuclei depend chiefly on the manner in which the chromatin is distributed. Two principal types of structure may be distinguished; in the first the chromatin is concentrated into a single mass or grain, or, if other grains are present in the nucleus, they are smaller and relatively insignificant in size; in the second a number of grains are present which are more or less equal in size. In the condition with a single, or one greatly predominating, mass of chromatin, the nuclear space is not as a rule filled by it, but presents the appearance of a vesicle containing the chromatin mass at or near its centre; consequently such nuclei are commonly termed "vesicular" in type, and the chromatinic mass may be termed generally, and without further determination of its precise nature, an *Endosome* ("Binnenkörper"). When, on the other hand, the chromatin is in the form of numerous grains, they are generally distributed more or less evenly throughout the nuclear cavity; such nuclei are termed "granular." '

It is the granular type of nucleus that is said to be one of the principal criteria distinguishing the entamoebae from the amoebae of the free living species. As far as my experience permits me to state, I am in accord with this differentiation. All the nuclei of the entamoebae which I have studied are certainly well differentiated from those of the free living amoebae that I have observed. Some authors, however, give drawings of nuclei of free living amoebae that resemble very closely those of the entamoebae, and Gauducheau (1912*b*) described transitional phases between the two types in amoebae from his cultures.

None the less, as far as *E. coli* is concerned, the nucleus may be taken as very typical of the granular type, and as such merits especial attention, not only for purposes of differentiation, but for biological study as well.

For the most part, in fresh preparations, the nucleus of *E. coli* is larger than that of the pathogenic species, and the membrane appears thicker. But in the *tetragena* type the achromatinic network, the stout, double-contoured membrane, and the central mass of chromatin, resemble closely enough what is seen in fresh preparations of *E coli.* Nor have I found that the nucleus of E. *coli* is constantly distinctly visible in fresh preparations. I have noted more than once, when in fresh preparations the nuclei were of all degrees of visibility, that in stained preparations made at the same time the nuclei were relatively prominent. In my opinion, in *coli*, as in the pathogenic species also, visibility of the nucleus depends upon phases of the life cycle and the stages of metabolism, and this sometimes when poorly-defined nuclei in fresh preparations show abundant chromatin when fixed and stained.

When the nucleus is plainly visible in fresh preparations, it shows rather abundant chromatin in the form of highly refractive granules, distributed over the surface, or under the nuclear membrane; a well-defined membrane; and a central mass of chromatin, circular, and of relatively large size. The nucleus very seldom changes its shape when passing between vacuoles, or when pressed between these or between the margin of the entamoeba and the cytoplasm, which is in contrast to the behaviour of the nucleus of the *histolytica* type. Werner notes that at times there is a narrow border of equal sized, highly refractive granules, surrounding the nucleus, often observed during life, but which he could not demonstrate plainly after fixing and staining. The same appearance is seen, but to a less degree and not so well defined, in the *histolytica* and in the *tetragena* types. It is due, very likely, to blocks of chromatin which are somewhat altered in position and size by fixing and staining reagents.

As Hartmann and Whitmore note, while the nucleus of *E. coli*, after fixing and staining, shows in general the same characteristics as that of the *tetragena* type, yet a thorough study will demonstrate a series of characteristic points of differentiation. These points do not appear so much in any one entamoeba as in several; in fact, the authors state that a large number must be observed. In such a study it is best to take up the component parts of the nucleus. As given by Minchin (1912) these are:—'(1) An achromatinic framework, or nuclear reticulum; (2) a true membrane, formed from the achromatinic framework, and separating the nuclear contents from the surrounding cytoplasm; (3) nuclear sap, pervading the entire nuclear cavity; (4) plastin, in the form of one or more bodies or masses which may consist of pure plastin (nucleoli) or of plastin impregnated with chromatin (karyosomes); and (5), the chromatin, which may be present either in the form of granules lodged at the nodal points of the reticulum, and scattered evenly or unevenly throughout the nuclear framework, or may be concentrated in a karyosome or may combine both of these two modes of distribution in various ways.'

1. *The achromatinic framework.* According to Minchin (p. 75) the apparent 'threads' of the achromatinic reticulum are but the optical expression of the walls or partitions separating alveoli, and there is no reason for considering the achromatinic reticulum or linin network as different in any essential point from the alveolar framework of the general protoplasm. As compared

with the alveolar structure of the general protoplasm, Minchin states, that of the achromatinic framework of the nucleus is characterized chiefly by the larger size of the alveoli, and, consequently, the greater distinctness of the apparent reticular structure.

This description as applied to the achromatinic framework of the nucleus of *E. coli* is, in so far as fresh preparations are considered, essentially correct. In these the network is made out in direct proportion to the visibility of the nucleus. It is not highly refractive, and appears as delicate interwoven threads. In the pathogenic species this network varies greatly in amount and structure, but when visible in any quantity, as in the *tetragena* type of nucleus, it is finer meshed and is more delicate than is usually seen in *E. coli*.

Depending on the stain used, this network is more or less well demonstrated. After wet fixation and iron-hematoxylin staining it is not very often plainly seen, but when visible, appears as rather stout threads of lighter colour than the chromatin, with thickening at the nodal points (figs. 57-69, 97-99). The iron-hematoxylin stains diffusely and feebly something else in the nucleus, probably the nuclear sap, or enchylema, and this diffusely-stained substance blurs the definition of the nuclear framework. But after Mann's stain, and after lichtgrün, carmine, and other substances that are good cytoplasmic dyes, the network is very well demonstrated (figs. 149, 151-154), and this upholds Minchin's contention that the achromatinic network is a cytoplasmic structure. After Mann's stain, however, the threads stain much more clearly and deeply than do those of the cytoplasm.

When there is but a single mass of chromatin in the nucleus, or when there is one mass much larger than the others, the reticulum shows as delicate threads radiating from the principal mass to the periphery. When, on the other hand, there are numerous grains of chromatin more or less equal in size, the reticulum is seen as fine lines passing from each grain to the adjacent grains. When the chromatin is condensed into a single central mass, with or without other masses at the nuclear periphery, the reticulum is wide meshed (figs. 149, 151), but when the chromatin is distributed at the nodal points of the network, or appears as several masses in the central portion of the nucleus, then the reticulum is closer meshed, with thicker threads, and the meshes become closer towards the centre (figs. 152-153). The reticulum of the nucleus of *E. coli* is in general coarser and more nodular than that of the nuclei of the pathogenic species. (Compare figs. 145-148 with 151-154).

During the cyclical changes in the nucleus, the reticulum sometimes appears in ring forms (figs. 151-152), as was noted by Werner (1911, p. 70), and again most of the reticulum is collected in the central zone of the nucleus (fig. 149), with a few threads only connecting it with the periphery.

After dry fixation and staining with Hastings and Giemsa, the reticulum is very often beautifully demonstrated (figs. 137-140), and with its relatively large amount and well-defined structure constitutes an important criterion of differentiation as compared with that of the pathogenic species after the same technique.

2. *The nuclear membrane.* This is, as noted, frequently visible in fresh preparations, when it is more distinct than in most phases of the pathogenic species, except in those of the *tetragena* type associated with acute dysentery. In stained specimens, for the most part the membrane is distinctly visible, and as a rule somewhat thicker than in the pathogenic species (figs. 57-69, 151-152). The thickness is variable, however, and often the membrane appears as a very delicate line, apparently thickened by the peripheral chromatin (figs 57, 58, 69, and 149, 154). As Minchin states: 'When there are numerous grains of chromatin, those placed superficially may unite to form a limiting layer which may be termed a "false" or "chromatinic" membrane, in distinction to a true nuclear membrane, which is an achromatinic structure.' Such an appearance is very deceptive (fig. 68), and has led, in my opinion, to a false conception as to the value of the nuclear membrane in the determination of species.

The membrane takes the same colour after Mann's stain, and other protoplasmic dyes, as does the nuclear reticulum, of which apparently it is a part.

3. *The nuclear sap.* This appears only after iron-hematoxylin staining, as a brown coloration of a part or a whole of the nucleus, and this colour varies in intensity in proportion to the degree of decolorization (figs. 59, 61, 68). It is very easily altered by fixing

reagents, and for this reason is varied in appearance. The same description as above applies also to the nuclear sap of the pathogenic species.

4. *Plastin*. I cannot state to what degree, if any, this substance is present in the nucleus of *E. coli*, or in those of the pathogenic species. Minchin notes that plastin is sometimes the matrix of the karyosome in the protozoon nucleus, especially in the vesicular type, but that nucleoli composed entirely of plastin, such as are found almost invariably in the tissue cells of metazoa, are not of common occurrence. Doflein (1911), under the title of 'Die Nucleolarsubstanz,' says that this substance is often identified as plastin, without particular reason, and states that it stains after nuclear dyes but feebly, and strongly after acid dyes.

After iron-hematoxylin a well-defined homogeneous central body is often made out in the nucleus, in which a central mass of more deeply staining chromatin is embedded (fig. 95), or the central mass of chromatin may be divided into several pieces, which are united by the feebler staining substance (figs. 60, 69). Again, the central chromatinic mass lies in a clear zone (fig. 57), around which is the achromatinic network. Between a more or less well-defined reticulum and the homogeneous mass such as is seen in fig. 95 are all degrees of variation, so that after staining with iron-hematoxylin it cannot be said in many instances which is condensed reticulum and which is something else.

After Mann's stain the chromatin is coloured orange (figs. 149, 151-154), and the reticulum blue. With this stain the plastin, if such it be, takes the same colour, but deeper, as does the reticulum (fig. 153). In the *histolytica* type of nucleus small central blocks of this blue substance are sometimes seen, with the orange-coloured centriole embedded in them, and after iron-hematoxylin a structure in varying shades of colour may be demonstrated (figs. 100-103), while in the *tetragena* type a well-defined central mass, made up of chromatinic dots and a homogeneously-staining material is often plainly discernible (fig. 22). Later this mass breaks up to a well-defined reticulum with nodal points of chromatin, the nuclear cycle of Hartmann (fig. 25*b*). After Mann's stain this central mass is at first almost homogeneous in the *tetragena* type of nucleus (fig. 147), and later in the nuclear cycle the well-defined reticulum is seen (fig. 148). Similar cyclical changes take place in the nucleus of *E. coli*, and will presently be described. However, if Doflein is correct in his statement that true plastin stains strongly after acid dyes, then this blue central body cannot be plastin, for if it were it should take the eosin of Mann's stain, and not the methyl-blue, as it does.

As far as I can determine, using Mann's stains as a guide, the nucleus of *E. coli* is made up of a central mass that takes in varying shades the methyl blue component of the stain, and embedded in this mass is more or less chromatin, taking an orange stain. During the cycle of the nucleus this mass breaks up, and a reticulum appears, which spreads from the centre of the nucleus to the periphery. At the beginning of the cycle this central mass corresponds to the 'endosome' of Minchin, and the 'Binnenkörper' of the Germans. It does not seem to me that it has any relation to the nucleoli, or plastin masses, of metazoan cells, since the latter take an orange colour after Mann's stain, as is plainly seen in those body cells thrown into the intestinal lumen during acute dysentery.

5. *The chromatin.* In the nucleus of *E. coli* the chromatin is found in exceedingly variable amounts, so that no one phase should be taken as typical of the nucleus of this entamoeba (figs. 57-69, 149, 151-154).

Hartmann (1909) described a cycle of the karyosome in the nucleus of *E. coli*, and later, in his joint paper with Whitmore (1912) the opinion is expressed that by careful attention to this cycle it is possible to distinguish between *coli* and *tetragena*, if enough organisms are studied. In this I agree with these authors, although Werner (1911) who also recognized in the nucleus of *coli* cyclical changes analogous to those in the nucleus of *tetragena*, is of the opinion that a differentiation cannot be made by such a study. In my hands, fixation with Schaudinn's fluid, and staining with Mann's, has proved the best method to demonstrate these changes. Dr. Elmassian, however, prefers fixation with Duboscq's fluid.

In the simplest form, which also occurs least often, the nucleus of *E. coli* shows in its central portion a round, compact mass of chromatin, staining a dense black after iron-hematoxylin (fig. 57), and a deep orange after Mann's. This mass is connected with the periphery by the reticulum, which at this stage is relatively open meshed, and shows few, if any, granules of chromatin at the nodes (fig. 57). In the primary stages of cyclical development, such as probably occur after binary division of a vegetative form, there is but little peripheral chromatin (fig. 57), but at the beginning of a second cycle the peripheral chromatin is usually abundant, and in large blocks (fig. 151).

The cycle begins with the breaking up of this central mass of chromatin, the karyosome of Hartmann and Whitmore, and also the chromatin increases in amount, while division of it is somewhat irregular in the vast majority of organisms (fig. 59). This irregular and unequal division (figs. 60, 64, and 99) is quite characteristic, as first pointed out by Hartmann and Whitmore. Sometimes division takes place by an apparent promitosis (fig. 98), and in the latter instance the central chromatin apparently functions as a centriole. To me it seems probable that only one method of division of the central mass leading ultimately to division of the entire nucleus actually occurs, so that the multipolar division such as is shown in fig. 64 is due to the throwing off of one or more blocks of chromatin which make their way to the periphery, and such breaking up is cyclical, while true nuclear division begins as is shown in fig. 98.

As the central mass begins to break up, the reticulum becomes closer meshed, with thicker threads, and grains of chromatin of various sizes are seen at the nodal points (fig. 153). All degrees of chromatin distribution over the network and along the periphery may be followed, until finally the chromatin is collected mostly in large blocks at the periphery, only a small granule remaining in the centre (fig. 149). How many times the cycle may be repeated before nuclear division takes place I do not know. Hartmann (1912) gives illustrations showing evidences of the cycle of a *tetragena* nucleus in its third repetition, and clear evidences of a second repetition can be obtained without difficulty in that of *coli*, but I have not seen more than this (fig. 151).

Except at the end stage of the cycle, the central mass of chromatin in the nucleus of *E. coli* is relatively and actually larger than that of the pathogenic species, and as such is an important mark of differentiation. Further, the irregular, and often large, blocks of chromatin resulting from the binary and multipolar breaking up of this mass (figs. 60, 64, 65), which are plainly discernible in a considerable number of the vegetative organisms (Hartmann and Whitmore give the number as from 20-30 to 50-70 per cent.), are in striking contrast to the minute and equal-sized particles formed by the breaking up of the central mass in the *histolytica* and *tetragena* types (fig. 108). As Hartmann and Whitmore insist, this appearance in *coli* serves as a principal criterion for the differentiation between this organism and the pathogenic species.

Although with this exception the differences between the nucleus of *E. coli* and the nuclei of the pathogenic species are of degree rather than kind, a careful study will bring out other criteria of value. The nucleus of *coli* is perceptibly larger, and often contains actually and relatively more chromatin than the nuclei of the pathogenic organisms. Double central masses, of the size shown on figs. 63 and 66, while common enough in *coli* infections, I have never observed in infections with the pathogenic entamoebae. After dry fixation, and staining with Hastings and Giemsa, the plentiful and well-defined nuclear reticulum with the relatively abundant erythrochromatin scattered over it in blocks and threads (figs. 137-140, 144) is in very marked contrast to what is seen in the *histolytica* and *tetragena* types of nuclei (figs. 124-129).

Yet even when all these points are considered, it is not possible at times dogmatically to say, with respect to nuclear morphology in the vegetative stage, that a certain entamoeba must belong to a certain species. For although the sum of the nuclear characteristics of each species is of highest differential value, many organisms individually present a nuclear morphology on the border line, and even in known mixed infections, it has not been my experience that I could invariably refer every organism encountered to its proper species. For example, in fig. 4, from a case of very acute dysentery, the nucleus resembles closely enough that of an *E. coli*, and the same is true in fig. 127, from the pre-cystic stage of a *tetragena* type infection.

And if this be true of the nuclear differentiation between two well-defined species of the entamoebae of man, what value should be attributed to ***nuclear differcntiation*** alone in the identification of doubtful species? Yet some species are claimed solely on the grounds that minute differences in the arrangement of the chromatin justify a distinction.

MULTIPLICATION IN THE VEGETATIVE STAGE
BINARY, OR SIMPLE DIVISION, AND MULTIPLE DIVISION, OR SCHIZOGONY

Casagrandi and Barbagallo first described multiplication of *E. coli*, giving an account of simple division of the nucleus and cytoplasm. Later Casagrandi and Barbagallo pictured various phases of binary division of the nucleus, and some of their drawings are reproduced by Craig (1911), Werner (1911), and Doflein (1911). These illustrations are apparently from living specimens, since Werner thus describes the two presented by him, and these are given also, with others, by Doflein. However this may be, the pictures of Casagrandi and Barbagallo do not give much information as to the mechanism of nuclear division, nor do they appear to me to be particularly definite representations of *E. coli*, either living or fixed and stained, especially as compared with the specimens stained by modern techniques.

According to Hartmann and Whitmore, Schaudinn stated that binary nuclear division in the vegetative forms takes place by amitosis. Craig (1911), however, states that after wet fixation and iron-hematoxylin 'clear evidences of a mitosis are frequently observed.' According to this authority the nucleus elongates, and the chromatin becomes concentrated at the poles. After the nucleus has become much elongated, a constriction develops near the centre, and division takes place. At the same time a constriction appears in the cytoplasm, and this constriction deepens until, after division of the nucleus, the entamoeba also divides, a daughter nucleus appearing in each half.

Doflein states that in binary division of the nucleus a mitosis of the karyosome is to be observed.

Hartmann and Whitmore, although they made an extended search through their preparations, could demonstrate only the beginning of nuclear division, as shown by division of the central mass of chromatin, which mass they call the karyosome, and the two-nucleated entamoebae resulting from binary division. Reasoning from their researches on the allied *E. tetragena* and *E. histolytica* species, they express the opinion that binary division takes place by a mitosis or by a promitosis.

Although I searched many preparations, representing many hundred vegetative forms, I could not demonstrate the complete phases of binary nuclear division. Had I more material representing very active vegetative increase, I feel sure that the complete phases could be found, but for the present I must leave this part of my research incomplete. Division of the karyosome, binary and multiple, I saw often, and sometimes as an apparent promitosis (fig. 98). Many nuclei contained two well-defined central masses, representing division of the karyosome, without further evidence of binary division (figs. 63, 65, 69, 99). Also such nuclei as are shown in fig. 66, which according to Craig would represent well-advanced division, were not infrequently met with. Fig. 68 may be interpreted in two ways: (1) as beginning division of the nucleus and the cytoplasm, with the enlarged karyosome about to divide, or (2) the constriction of the nucleus is accidental or mechanical, the latter as a result of cytoplasmic constriction. I regard the second interpretation as more probable, since such specimens were very infrequently encountered. Figs. 59 and 61 show elongated nuclei; in fig. 59 the central mass is much enlarged.

However, such observations as I have made lead me to the belief that binary nuclear division is mitotic or promitotic, rather than amitotic, especially since in the cysts very beautiful promitoses may be seen. The problem will be solved as soon as enough work is done on suitable material, which is difficult to obtain in any quantity.

The scanty number of individuals of *E. coli* that show what may be interpreted as later phases of binary nuclear division, especially when the entamoebae are present in any number and two-nucleated forms are relatively frequent (as is true in a number of my preparations), is in marked contrast to what is seen in the vegetative forms of the pathogenic species during the stage of acute dysentery. At this time, if the organisms are plentiful, abundant phases of a mitotic or of a promitotic division can be demonstrated, and all phases of binary nuclear division followed. Yet, in the pre-cystic and cyst-forming generations of the *tetragena* type, although two-nucleated individuals may be plentiful, one sees but seldom mitotic phases of the vegetative forms.

Multiple nuclear division of the vegetative organisms, or schizogony, was described first by Casagrandi and Barbagallo, and later by Schaudinn. Doflein (1911) quotes Schaudinn:

> 'The nucleus swells by reason of taking up fluid, the cytoplasm (Weichkörper) frees itself from all foreign bodies and loses motility. The staining substance of the nucleus is divided into eight large peripheral blocks which, after the dissolution of the nuclear membrane, will be distributed to the plasma as daughter nuclei. At the same time the cytoplasm divides, corresponding to the number of the nuclei, into eight little amoebae, which creep away in different directions (p. 575).'

It has been impossible for me to obtain any satisfactory confirmation of this method of schizogony, either in my own work or by consulting that of others. The eight-nucleated vegetative forms pictured by Casagrandi and Barbagallo, and reproduced by various authors as above mentioned, leave one in doubt as to whether nuclei or something else are illustrated. Brown (1910), although he gives a minute description of such a schizogony, presents only a schematic illustration, and does not state whether the process was confirmed by personal observation. Craig (1911) states that he could follow almost every stage described by Schaudinn:

> 'In the living specimen the daughter nuclei are visible as highly refractive masses of granules due to the chromatin of which they are largely composed, while in stained specimens the chromatin takes on a light red colour when Wright's method is employed (p. 102).'

In Craig's Plate VII, however, the eight nuclei of the schizont are indistinguishable from the nuclei of cysts in the end stage, and the oval shape of his fig. C is at times found in undoubted cysts.

Doflein (1911) accepts without further comment Schaudinn's account.

Werner (1911) states:

> 'I have observed two eight-nucleated forms, which I may lay claim to (ansprechen möchte) as the end stage of this process of division (p. 71).'

But these figures of Werner's, his Nos. 13 and 14, are in no way convincing to me. They may very well be enucleate entamoebae (such as I shall presently describe) in which included cocci or other micro-organisms are represented as nuclei. My own fig. 67 shows such inclusions that resemble very closely the nuclei in Werner's figures, and if the nucleus has been entirely instead of partially hidden, or had it been absent, as is common enough, my fig. 67 and Werner's figs. 13 and 14 would be morphologically identical.

Hartmann and Whitmore (1912) state:

> 'With respect to these two forms of Werner's, it seems to us not certain whether they are entamoebae (p. 185).'

In my opinion the forms in question are entamoebae, but they are more than likely enucleate entamoebae in which inclusions have been mistaken for nuclei.

Prowazek (1911) gives a wet fixed and iron-hematoxylin stained representation of schizogony in *E. williamsi*, in his fig. 6, showing six nuclei. Yet the nuclei in this figure are identical with those in my figs. 90-92, which show the end stage of cyst-formation, while the cytoplasm in Prowazek's figure most certainly is of the type found in cysts rather than in vegetative forms.

The important recent paper of Hartmann and Whitmore (1912) throws much light on the problem of schizogony in *E. coli*. The beautiful illustrations by Luise Hartmann, the accuracy of which will be recognised at once by anyone familiar with the technique employed, enable one to follow very closely the authors' description of the process. In their observations, which were made on wet fixed and iron-hematoxylin stained preparations for the most part from material collected by Whitmore in Manila, they found what they interpret as vegetative forms with two, three, four, and six nuclei, and these nuclei they state were similar to those found in the one- and two-nucleated vegetative entamoebae, and their recognition of this point is, as far as I know, the first recorded in the literature. As will presently be pointed out, some of the end stages of cyst-formation in *coli* are quite similar to vegetative organisms in structure, and to prove an eight-nuclear schizogony it is necessary to show beyond doubt that cysts are not in question.

In support of their belief, Hartmann and Whitmore show two-nucleated vegetative entamoebae in which the nuclei show signs of commencing division. A four-nucleated organism, in the vegetative stage, is then shown, and additional pictures representing a three-nucleated and a six-nucleated vegetative stage are given. The authors do not present pictures of an end stage showing eight nuclei, nor is any mention made in their text of the finding of such a stage.

Further, they state that the collection of blocks of chromatin on the nuclear membrane, which was taken by Schaudinn as an indication of the breaking down of the nucleus into daughter nuclei, has nothing whatever to do with schizogony. Such blocks, they claim, are the product of cyclical changes in the nucleus, and are seen also in the two-nucleated vegetative forms of *tetragena*. In the authors' opinion, Schaudinn reasoned from an analogous reproductive process in *Calcituba*, when he described schizogony in *E. coli*. Hartmann and Whitmore do not describe nor picture two-nucleated vegetative forms of *E. coli* with large peripheral blocks of chromatin under the nuclear membrane, nor indeed do they give any such representation for the one-nucleated entamoebae, such as is shown in my fig. 151. They state, however, that the cyclical changes of the nucleus in *E. coli* are similar to those in *E. tetragena*, and this statement possibly includes the finding of the large peripheral blocks described by Schaudinn.

With Hartmann and Whitmore's objections to Schaudinn's description of schizogony in *E. coli* and to the confirmation by other writers of this description, I fully agree, for the reasons given above. Not only in two-nucleated vegetative forms of *tetragena* is the chromatin arranged as Schaudinn described it for schizogony in the one-nucleated vegetative forms of *coli*, but similar arrangements can be demonstrated in the two-nucleated cysts of *coli* as well (fig. 80). Probably further research will show two-nucleated vegetative forms of *coli*, with the nuclear chromatin arranged as in fig. 151, although as yet I have not found such forms, nor have I found them described elsewhere.

I believe, however, that further confirmation is necessary before it is definitely proved that an eight-nuclear schizogony takes place in *E. coli*. Accounts of this in fresh preparations may well be viewed with suspicion. Many vegetative enucleate forms are found in some preparations, and when such forms have phagocyted inclusions such as are represented in figs. 63, 152, 153, in fresh preparations the inclusions may easily be mistaken for nuclei, as I know from personal experience. For instance, in the stool from which preparations containing the entamoebae shown in figs. 63, 152, 153, were made, there was a large number of enucleate entamoebae, and while some of these were stuffed with the inclusions, others presented them in number from two to eight, and in the fresh preparations the inclusions resembled very closely nuclei, to me more closely than do the drawings of Casagrandi and Barbagallo showing schizogony in fresh preparations. Moreover, in the past not a few workers have interpreted various phases of intranuclear and cytoplasmic parasitism in certain protozoa for reproductive changes.

However, there is no question but that true nuclei are represented by Hartmann and Whitmore in their description of schizogony. The question now arises, had these observers to do with a true schizogony or with phases of cyst formation? Major Whitmore, in a personal communication, informs me that this problem was very carefully considered in each case, and points out that the figs. 5, 6, and 7 of Hartmann and himself, which show respectively four, three, and six-nucleated vegetative forms, have the same cytoplasmic structure as do their figs. 1 and 2, which are plainly enough vegetative organisms, but not the same cytoplasmic structure as their fig. 18, a two-nucleated cyst. Major Whitmore further called my attention to the cytoplasmic structure of their figs. 5, 6, and 7, as containing in each instance food vacuoles with masses of food in various stages of digestion, and pointed out that one of the first steps in cyst-formation is the throwing out of such food masses.

Now, as I shall show subsequently, there are two, if not three, kinds of cysts of *E. coli*. One of these, after staining with iron-hematoxylin, has a cytoplasmic structure very similar to that of the vegetative organisms (figs. 93, 94). These preparations were made from a solid stool, in which there were no vegetative organisms, so they are without doubt cysts. But after Mann's stain organisms of this kind take on a characteristic colour, which serves to differentiate them from vegetative entamoebae (figs. 154, 156). After iron-hematoxylin staining, in the various phases of cyst-formation, there is more than a superficial resemblance between this class of cyst and the vegetative forms, especially those shown in Hartmann and Whitmore's figs. 5, 6, and 7. Indeed, the nuclei shown in their fig. 7 (that of the six-nucleated schizont), resemble very closely the nuclei in my figs. 80, 156, and in their own figs. 23 and 26, which represent, as do my figs. 80, 156, stages of cyst-formation.

Unfortunately, cysts of the type shown in my figs. 80, 84, are seldom found, and I could not complete a series of such cysts from the one-nucleated stage to the eight.

These facts, as just presented, to me afford sufficient grounds for waiting until further work has been done before accepting an eight-nuclear schizogony. When a definite and indisputable series of illustrations will have been presented, showing the entire process, the hypothesis may be considered as substantiated, but from my point of view it is not substantiated in the light of our present knowledge. I have examined very many preparations from all phases of *coli* infections, in health and in disease, and in latent and in very active cycles, but I have not found any proof of schizogony. At the same time the work of Hartmann and Whitmore emanates from authorities whose conclusions may not lightly be criticised, and my failure to confirm their conclusions in this respect is certainly no reason to doubt their correctness. Some of the entamoebae that I consider cysts may be vegetative organisms, especially fig. 156, and since schizogony of *E. coli* is admittedly a process that occurs but very seldom, I may very well not have found it in my specimens.

FREE NUCLEI AND ENUCLEATE ENTAMOEBAE

In 1882, Grassi, according to Elmassian (1909*a*) observed the presence of free nuclei, and of protozoa without nuclei, and stated that this occurred frequently among the *Amoebae*. Grassi's opinion was that after binary division of the nucleus, one of the daughter nuclei was extruded. Later, cytoplasm formed around the extruded nucleus, and a normal amoeba developed. Elmassian (1909*a*), working with *E. blattae*, the entamoeba of the cockroach, observed numerous free nuclei, in perfectly normal condition, and many enucleate entamoebae, also in a normal state, except for the absence of the nucleus. Elmassian also observed expulsion of the nucleus in the living organism. He noted that this is a common phenomenon in *E. blattae*, but stated that he could not give an adequate explanation. 'If such extrusion had not been so common,' he states, 'I could have believed that I had to do with an exceptional pathogenic occurrence.' Mercier (1910), who also observed these free nuclei, believed that their occurrence resulted from the destruction, by various micro-organisms, of the cytoplasm of the entamoebae, since Chatton and Brodsky (1909) had previously noted, in one of their cultures of *A. limax*, the persistence of the nucleus after destruction of the cytoplasm. According to a later work, Chatton and Brodsky (1910) stated that the amoebae disintegrated as a result of the intracellular increase in the number of a certain cytoplasmic protozoal parasite, and later the cytoplasm, or rather what remained of it, was destroyed by bacteria.

Extrusion of the nucleus in any of the entamoebae of man has been noted but seldom, and then under artificial conditions. Schaudinn observed this extrusion during the stages of the so-called spore formation in *histolytica*. Darling (1913*c*) found a number of free nuclei in a mature strain of *E. tetragena*, during the fourth passage through kittens. I found it in moist chamber preparations of the vegetative forms of the pathogenic entamoebae. I have not found, however, any report of the occurrence of this phenomenon in *E. coli*, nor in preparations of the pathogenic species from untreated cases in man, when the preparations were made immediately after the stools were passed.

In only one of my cases of infection with the pathogenic species have I observed extrusion of the nucleus in specimens fixed immediately after the stool was passed. In preparations from this case, which contained vegetative forms of the *tetragena* type in large numbers, there were many free nuclei, and also many entamoebae whose cytoplasm was normal after all my fixing and staining reagents, but without nuclei. No budding forms were found, nor did I observe any of the phenomena of nuclear disintegration and chromidia formation, as described by Schaudinn and by Darling. In this case the nuclear extrusion appeared to be analogous to what takes place in *E. coli*.

An infection with *E. coli*, which is known to have persisted for over five years, has been under my observation for several months past. Whenever vegetative entamoebae were present in the stools in either large or small numbers, free nuclei were found, and also enucleate entamoebae. Sometimes the percentage of enucleated organisms was quite high, in one set of preparations 15%, and free nuclei were present in proportion. In this infection the extrusion of these nuclei does not seem to bear any definite relation to any particular phase of the life-cycle, since enucleate entamoebae were found in all stages of the cycle of the vegetative entamoebae, up to cyst formation, and even in cyst formation some organisms with definite cyst cytoplasm did not show nuclei. The free nuclei were for the most part normal, and differed from included nuclei only in that the latter were somewhat smaller (figs. 95, 149). In many of my preparations a morphological series, showing intermediate stages between free nuclei and well-developed entamoebae, could be demonstrated, thus lending apparent confirmation to Grassi's hypothesis (figs. 95-99). Personally, however, I believe that this evidence is morphological, and not biological, and as such should be regarded with suspicion.

In cytoplasmic structure the enucleate entamoebae differed in no manner from those with well-formed nuclei. There were as many degenerating entamoebae with nuclei as without them. Fig. 62 shows an enucleate entamoeba in very good health, as shown by its activity, its cytoplasmic structure, and its inclusions. I may add that for every representation of a nucleated vegetative entamoeba shown in my plates, it was possible to demonstrate enucleate organisms entirely similar as to cytoplasmic structure and inclusions.

Parasitism of the entamoebae, as described by Chatton and Brodski, can be dismissed as a factor of any importance in the consideration of the phenomenon as presented in my specimens, since the phenomenon occurs without regard to the number of inclusions. Extrusion is not essentially associated with any physiological degeneration, such as is seen in the pre-cystic and cyst-forming generations, for it was observed in phases of the cycle when pre-cystic entamoebae and cysts were absent from the preparations. Nor could I trace any particular degeneration in either the free nuclei or the enucleate entamoebae, since by far the greater number of each were entirely normal in the phases of the vegetative organisms, and in the pre-cystic and cyst-forming generations very many degenerating entamoebae are always found.

Extrusion of the nucleus is, in my experience, by no means met with in all infections, since I observed it to any extent in only one of over fifty pathogenic infections, and in only two of twelve *coli* infections. The phenomenon as I have described it must not be confused with the nuclear extrusion described by Schaudinn and other writers for the pathogenic species, for the latter extrusion has, as I shall later point out, an entirely different significance, and is directly concerned with degeneration of the entamoebae. I could not find extrusion in any living organisms, and I cannot state if fig. 67 represents actual extrusion, similar to that described and pictured by others, or an accidental occurrence. Extrusion, as I have described it, I do not believe to be an artificial phenomenon, since the preparations were made immediately after the stool was passed, and, moreover, it should have been more common if it were artificial, unless the entamoebae were in some physiological condition that permitted this extrusion to take place during the few moments required to spread the specimens prior to fixation. Even then, it would be remarkable that such a physiological condition should exist through several cycles and over a period of several months. I have not found any satisfactory explanation of this phenomenon in the writings of others, and while I have none to offer myself, I present this description on account of the biological interest that an unexplained phenomenon in the cycle of the entamoebae should call forth.

CYST FORMATION

Cyst formation in *E. coli* follows along the same lines as in the pathogenic species. After a period of active vitality the entamoebae become smaller; the cytoplasm frees itself from inclusions, and in fresh preparations becomes highly refractive, while an external membrane is formed from the ectoplasm. The relative ease with which cyst formation can be followed in *E. coli* renders this organism highly suitable for the study of one of the most interesting phases in the life-cycles of the obligate protozoa of man, that of the formation of bodies which will continue life outside of the intestinal tract. Schaudinn, quoted by Brown (1910), stated:

'In spite of the complexity of the processes of sexual reproduction, I know of no cell more suitable for nuclear study than the encysted stage of *E. coli.*'

In the pathogenic species encystment is preceded by a well-defined pre-cystic generation with the formation of chromidia. The process occurs only when the stools are no longer dysenteric in character, that is, when they are becoming formed. In most instances there is, as has been noted, what has been designated as an 'epidemic' of cyst-formation, so that pre-cysting, cyst-forming, and degenerating entamoebae are encountered in great profusion.

Now most infections with *E. coli* happen in persons whose intestines are normal. In formed and in solid stools only cysts are found, but when the faeces are semi-solid, vegetative organisms are met with, and all degrees of cyst-formation and of the vegetative cycle may be observed.

But when there is diarrhoea, so that the stools are very loose, only vegetative entamoebae are found. So that in *E. coli*, as also in the pathogenic species, the formation of solid stools is a definite factor in encystment, although to what extent I have not as yet determined. It is, of course, obvious that since the vegetative entamoebae need for existence a liquid or a semi-liquid medium, the relatively dry material in formed stools constitutes an environment unsuitable for life, and a change of structure is imperative. But whether the entamoebae will encyst at any period of their vitality, or whether encystment takes place only at certain periods, cannot as yet be stated with certainty. I have repeatedly encountered negative findings in the solid stools of patients to whom the administration of saline or other cathartics resulted in demonstration of numerous vegetative entamoebae. Moreover, the appearance of a very large number of vegetative organisms in such liquid stools was not always followed by an 'epidemic' of cyst formation. Frequently the cysts were very scanty, and sometimes could not be found.

However, in favourable instances, such as during the slow subsidence of an artificial or of an acquired diarrhoea, a uniformity of the phases of the cycle in *E. coli* is observed, corresponding to that seen in the pathogenic species during recovery from acute dysentery.

First of all, there is a well-marked pre-cystic generation. In fresh preparations the entamoebae become more homogeneous in structure, and more refractive. In organisms which have lost their progressive motion, an alternate contraction and expansion of the endoplasm is plainly discernible, during which included particles are expelled. Some entamoebae are homogeneous throughout, while others show a definite granular centre, with a very broad periphery of homogeneous refractive substance. All degrees of visibility of the nuclei are observed, and where these are invisible, they can be demonstrated by vital staining. External membranes, some very delicate, and others stout, refractive, and double-contoured, are seen in optical section.

In preparations dry fixed, and stained with Hastings and Giemsa, the entamoebae stain very deeply, taking a dark purple colour (figs. 140-141). Such appearances are quite characteristic of *E. coli*, as compared with similar phases of the pathogenic species (figs. 127-129).

It is, however, in wet-fixed preparations that the changes in cyst formation are most typical and most easily followed. The cytoplasm becomes less vacuolated and more alveolar (figs. 59, 64). It stains intensely, and it is often difficult to obtain proper differentiation. With Mann's stain the cytoplasm takes on a very typical appearance. The colour changes from the delicate lilac of the vegetative organism to purple, and often to a very deep shade. Fig. 154 represents an entamoeba in the primary stage of encystment. The external cytoplasm is markedly alveolar, showing also fine granulations at the intersection of the alveolar walls, while the centre stains deeply, and is still vacuolated. This differentiation into a wider-meshed periphery and a closer-meshed central portion sometimes persists until the end stage of cyst formation (Text-fig. E), and except in the very latest stage the nuclei are always found in the denser portion. Text-fig. C shows a four-nucleated cyst of this kind.

Some of the pre-cystic entamoebae present appearances strongly suggestive of chromidia formation (figs. 70-72). These appearances are strikingly similar to those described for the pre-cystic stages of the pathogenic species, particularly the nuclear alterations. The inclusions stain similarly to the chromidia of the pathogenic organisms after all of my reagents, and in this respect cannot be differentiated.

Fig. 73 shows what may be taken for expulsion of included material, and fig. 75 represents an organism apparently at the beginning of encystment, with part of its membrane very attenuated.

All entamoebae that I observed in the pre-cystic stages were uninuclear, although the appearance of the central mass of nuclear chromatin in figs. 59 and 64 suggests a beginning division.

The further morphology of cyst formation has been variously interpreted, and at the present is a subject of much discussion among those who make a study of the entamoebae. Reasoning from analogy, Schaudinn described a very complex process of self-fertilization, or autogamy. Later, Hartmann and Whitmore (1912) insist that nuclear development within the cyst follows solely from simple division of the primary nucleus, thrice repeated.

Werner (1911) goes into detail with respect to Schaudinn's description of the nuclear changes in cyst formation, and the following is abstracted from his account (pp. 72-74).

According to Schaudinn, the primary cyst nucleus divides by a primitive mitosis, and two daughter nuclei result. These nuclei proceed to opposite poles of the cyst, and meanwhile a clear space* is formed, dividing the cytoplasm into two parts. Werner notes that this formation of a clear space is by no means universal. He states that the clear space does not stain by any of the reagents which demonstrate chromidia, and evidently is a vacuole. He then continues Schaudinn's description.

In the living cyst the two daughter nuclei now become less visible, and may disappear, but after a time they will again become plainly visible. Staining shows that this diminution in visibility corresponds to a stage in which the peripheral chromatin of the nuclei is thrown out into the surrounding cytoplasm. This giving up of the chromatin may be only partial, or the metamorphosis of the nucleus may be entire.

Later the nuclei are reconstructed, and this follows in several ways. A part of the original nucleus remains, and is used for reconstruction, or a part of the extruded chromatin is similarly used. The chromatin used in reconstruction is the 'generative nuclear substance (Geschlechtskernsubstanz),' while that which is not used, the 'degenerative nuclear substance (Stoffwechselkernsubstanz)' is taken up by metabolic assimilation, or else is expelled from the cyst. Whether this 'Stoffwechselkernsubstanz' remains in the cyst, or whether it is expelled, depends on the degree of hardening of the cyst wall. If the cyst wall is soft, expulsion takes place; if the cyst wall has hardened before the nuclear reconstructive processes are completed, the 'Stoffwechselkernsubstanz' remains in the cyst.

---

*This 'clear space' is now interpreted as a vacuole.—W.M.J.

Each of the two reconstructed nuclei now divides, the first reduction division, and of the four nuclei that result from this division, two persist, and two degenerate. The two nuclei that persist now divide, the second reduction division, and of the four nuclei that result from the second division, two persist and two perish, leaving in the cyst after this second reduction division two persisting nuclei and the remains of four degenerating nuclei. In the meantime the vacuole has decreased, and has vanished by the time the second reduction division has been completed, so that the two nuclei remaining after reduction and reduction division have been completed may be differentiated from the two nuclei resulting from primary division of the cyst nuclei, in that the former occur in cysts without vacuoles, while the latter occur in cysts with vacuoles.

Each of the two persisting nuclei now divides by mitosis and spindle formation, and four daughter nuclei result. These daughter nuclei are in pairs, at opposite poles of the cyst. These pairs are formed as follows:—One end of each spindle is stationary, while the other end, during the process of spindle formation, moves across the cyst. After division, that part of the spindle which did not cross the cyst forms the 'stationary nucleus,' while that part which crossed the cyst is known as the 'wandering nucleus.' Each pair of daughter nuclei is made up of a wandering nucleus and a stationary nucleus. The nuclei of each pair now fuse, and for a third time the cyst presents two nuclei. Each of these nuclei divides, and each of the four daughter nuclei divides, forming in this last division an eight-nucleated cyst, which in most instances is the end stage. Fertilisation is stated to be brought about by the union of the stationary nuclei with the wandering nuclei, and technically this is termed a double autogamy.

Wenyon (1907) described a somewhat similar process in the cysts of *E. muris.* He states that the vacuole, or 'clear body' as he calls it, persisted after the reduction divisions. The latest two reduction nuclei lay side by side at one pole of the cyst, and two spindles were simultaneously thrown across the vacuole. After division, the paired nuclei fused, so that each stationary nucleus united with the other, and each wandering nucleus with the other. Wenyon also noted two classes of cyst formation, one in cysts with vacuoles and the other in cysts without vacuoles.

Werner noted that the chromidial masses may be found in abundance in the one-nucleated cysts, as may also the vacuoles.

Hartmann and Whitmore, in a very thorough account of the process, note that cyst formation as described by Schaudinn has received but little attention from later writers, who have contented themselves mostly with picturing cysts with varying numbers of nuclei. Wenyon's work is taken up, as are the accounts of autogamy in *E. minuta*, by Elmassian, and in *E. williamsi*, by v. Prowazek. These two species, Hartmann and Whitmore say, are identical with *coli.*

As a result of their work, the authors come to the conclusion that no autogamy takes place within the cyst, but simple division of the nuclei only.

They state that primary division of the cyst nucleus takes place with and without the formation of a vacuole, as was noted by Wenyon and by Werner, and they show in their plates both methods. In the cysts with vacuoles there is a decided chromidia formation (Chromidienbildung), while in those without vacuoles this is not so marked. They argue from this that there is some relation between the occurrence of the vacuole and the amount of chromidia formed from the nucleus. They say that they have observed only the beginning of primary division of the nucleus. In the cysts with vacuoles, at first the chromidia formation is scanty, but in the two and four-nucleated forms it may be so plentiful as to obscure the outline of the nuclei.

After the four-nucleated stage is reached the vacuole disappears, and also, in most instances, the chromidia. If very many chromidia form while the cyst wall is hardening, some of them may remain in the cyst until the eight-nucleated stage. The authors consider that these are true chromidia, not reserve food bodies of cytoplasmic origin, because the chromidia react in a similar manner after the same reagents used to demonstrate chromidia in *tetragena* cysts.

After the four-nucleated stage, the nuclei again divide, simultaneously or irregularly, until the eight-nucleated stage is formed.

In most of Hartmann and Whitmore's preparations the eight-nucleated stage marks the conclusion of nuclear development within the cyst. However, in some of the *coli* infections from Manila they found large cysts, with 13, 15 and 16 nuclei. Since in undoubted *coli* infections, such as have been reported in Europe by Casagrandi and Barbagallo, and by Schaudinn, cysts with more than eight nuclei were found, the authors are of the opinion that the finding of cysts with more than eight nuclei, in infections in which the entamoebae otherwise are similar to *coli*, is not a certain criterion for differentiation, and so v. Prowazek's *E. williamsi* is not a true species. A sixteen-nucleated cyst, in their opinion, means that for some reason in the normal eight-nucleated stage each nucleus undergoes another division, and therefore cysts with nuclei in number between eight and sixteen will represent phases of such division.

After stating their own findings, Hartmann and Whitmore apply them to a criticism of autogamy, as described by Schaudinn and others. They claim that while in some cysts there is a heavy formation of chromidia, yet during the process the nucleus always remains fully individualized, as Wenyon and as v. Prowazek had claimed. Also, in some of the cysts there is no chromidia formation at any stage of nuclear division, which fact tells against Schaudinn's hypothesis.

They then quote Schaudinn's description of the reduction divisions of the nucleus and the subsequent union of the paired nuclei, an account of which I have given in the above abstract from Werner's work. They reiterate that the vacuole has no significance in nuclear division, since, as noted, this follows in the same manner with and without the forming of vacuoles. Two spindles stretching across the vacuole have then no meaning as a fertilisation process, but are no more than mitoses of the daughter nuclei after primary nuclear division.

Hartmann and Whitmore have also noted nuclei whose appearance corresponds closely to Schaudinn's description of the degenerating reduction nuclei. But the disintegration of degenerating nuclei is, they claim, found in different stages of cyst formation, as was shown in Hartmann's account of *E. tetragena.* They picture a cyst with three nuclei, their fig. 16, one of which is normal, while the other two call to mind reduction degenerating nuclei, but in reality are merely degenerating nuclei without relation to any process of reduction. Moreover, this cyst has no vacuole, whereas according to Schaudinn, the reduction process takes place in the vacuolated cysts. Also the reduction nuclei described by Elmassian and by v. Prowazek are merely degenerating nuclei that have no relation to a reduction process. v. Prowazek (1911), in his fig. 15, shows two nuclei, the one above the other, and this position he interpreted as a phase of conjugation. Hartmann and Whitmore point out that the position may represent equally as well a single nucleus immediately prior to complete division, or merely adjacent nuclei in the same line of vision, the more so since in cysts with great central vacuoles the nuclei are often tightly pressed together in the narrow rim of cytoplasm.

They claim that Wenyon's pictures of nuclear reduction and of copulation nuclei, in stained preparations, are subject to the same criticism. But Wenyon states that he observed much of the process in a single living cyst, and he gives pictures representing this. With respect to this, Hartmann and Whitmore state that the only one of Wenyon's pictures which could be interpreted as a fertilisation process (Wenyon's fig. 14, showing the paired nuclei prior to conjugation), may be interpreted also as four nuclei resulting from direct mitosis. The disappearance and reappearance of the nuclei (the reduction processes) is not proof of reduction and reconstruction, since by reason of the difficulty of observation two nuclei might readily become invisible for a while. True blending of the paired nuclei, as well as the following division into four, has not yet been observed by Wenyon in the living cyst.

These facts are the grounds for Hartmann and Whitmore's belief that no autogamy takes place within the cyst. They are of the opinion that fertilisation takes place by the copulation of the little entamoebae which come out from the cyst after this breaks up, subsequent to ingestion, in the intestinal tract.

ENCYSTMENT

Although, as noted above, I cannot state with certainty the precise relation between hardening of the stools and encystment of the entamoebae, I am of the opinion that encystment is primarily in direct relation to certain modifications in the vegetative organisms. Such modifications are manifested by an exhaustion of vitality, necessitating a change from reproduction by simple division, if the organisms are to survive, and may be brought about by external conditions that react on the entamoebae. Exhaustion of vitality is shown by the many degenerating forms encountered, especially the pre-cystic and encysting stages. The ability to encyst is in some manner inherent in the surviving organisms. Unless the entamoebae have attained that phase of vegetative life in which encystment is about to supervene, in an unfavourable environment the organisms perish. This can be demonstrated in two ways:—

1. If specimens containing only vegetative organisms which show no signs of encystment, such as may be obtained in acquired or artificial diarrhoeas, are placed in a moist chamber, the entamoebae die rapidly. But if in a certain proportion of the organisms encystment is taking place, the process will go on to the end stage in the moist chamber, except for such encysting organisms as degenerate before this stage, while the purely vegetative entamoebae die.

2. As noted above, if hardening of the stools takes place suddenly while the entamoebae are in the purely vegetative stage, no cysts will be found for some time, although the entamoebae may have been plentiful in the preceding liquid or semi-liquid stools. But when the stools harden gradually over a period of several days, it will be noted that in proportion to the number of vegetative organisms which show the characteristic morphology of beginning encystment, cysts will be found in the solid stools. Sometimes very

many vegetative entamoebae are found when the stools begin to harden, and later a few cysts only can be demonstrated, degenerating organisms being encountered in large number, while at others most of the entamoebae early show indications of encystment, and very many cysts are met with subsequently.

Solidity of the stools, while undoubtedly an important factor in encystment, is very probably a mechanical one, since in very loose stools passed for several days cysts in all phases can be found, provided that the vegetative organisms have attained that phase of the life cycle in which encystment must intervene to prevent degeneration.

In the light of our present knowledge it is obvious, from the long-continued persistence of some *coli* infections, that certain of the entamoebae are endowed with a very great potential of vitality, and these, in the course of multiplication by simple division, give rise to two strains, one of which persists and continues the infection in the intestinal tract, while the other at intervals goes on to encystment, to continue the infection outside of the body.

It may be, in these infections of long standing, that autoinfection from the cysts is the essential factor in the continuance of the infection, not certain of the entamoebae endowed with a great potential of vitality, but of this I have no direct proof. Neither have I any proof of conjugation of the vegetative entamoebae. In default of such proof I must for the present accept the hypothesis that the infection is continued by simple division of the vegetative entamoebae, while under certain conditions, the mechanism of which is entirely obscure, a part of the entamoebae goes on to encystment. The recent experiments of Woodruff (1912) with *Paramecium* indicate that in a favourable environment the original potential of vitality may continue unaltered through many thousand generations, without any necessity of conjugation to renew it.

The essential phenomena of encystment are:—

1. A change in the cytoplasm, which becomes alveolar, and then granular, during which change the inclusions are extruded (figs. 59, 64, 73, 84, 90), and, in many organisms, the appearance of a large vacuole.

2. The formation of an external membrane, which may be single or double in optical section (figs. 77, 79, 87, 88, 91).

3. Repeated division of the primary nucleus, until eight, and sometimes sixteen, nuclei are formed (figs. 76-94).

These phenomena do not follow in a regular and ordered manner, but vary individually as well as in their sequence. Further, as noted above, not all cysts go on to the end stage, since degenerating forms of these are encountered at any phase of the cycle of encystment. Attempts to classify as a definite sequence phenomena due to individual variation have led to much confusion in the interpretation of the appearance observed in cyst formation, and not until Hartmann and Whitmore (1912) published their work was the process of encystment clearly defined.

When an entamoeba is about to encyst, a very definite alteration in its cytoplasmic structure is observed, which I have described above. Further changes are as follows:—

(*a*) In some of the entamoebae the cytoplasm early divides into an alveolar periphery and a more granular centre, which type may persist through all the phases of nuclear division, as previously noted, while the nuclei remain in the granular portion until the end stage (Text-figs. C, D and E, and fig. 154). Such cytoplasmic structure is in most cases in relation to the formation of a cyst membrane, which for the most part is single and of delicate structure. After the end stage is attained, the cytoplasm becomes uniformly alveolar (fig. 90), and may be retracted regularly (figs. 91, 92) or irregularly (fig. 155) from the periphery, leaving between a homogeneously staining margin.

A similar separation of the cytoplasm into a granular and an alveolar zone has been described by Elmassian (1909*a*) and Mercier (1910) in the encystment of *E. blattae*. Both authors state that it is in relation to the formation of the cyst membrane. Mercier states that the granular portion, which encloses the nuclei, is in the central part of the cyst, while Elmassian describes this granular portion as at times irregularly distributed throughout the cyst, but always enclosing the nuclei. In *E. coli* quite frequently the granular cytoplasm occupies most of the cyst, the clearer portion appearing as a peripheral segment (Text-figs. C and D).

In a second type of cyst formation the cytoplasm is alveolar throughout. Such cysts are uniformly larger than the preceding, and the limiting membrane appears at varying intervals, and is always delicate. In such cysts nuclear division, as will be noted, is beautifully defined. I could not trace a complete developmental cycle in these cysts, but phases of it are shown in Text-fig. B, and figs. 80, 84, 88, 93, 94 and 156).

(*b*) A marked variation from the preceding types is found in the vacuolated cysts. Instead of an early separation of the cytoplasm into granular and alveolar divisions a large vacuole forms within the cyst, occupying most of the central part, while the cytoplasm is condensed at the periphery. Although I cannot state definitely the mechanism of the formation of the vacuole, I can bring forward certain facts that are invariably in relation, in my experience, to its appearance within the cyst.

In the cycle of encystment, the vacuole appears at a definite time. In all uninucleated cysts that show vacuoles, the nucleus is always already in division, never in the resting stage (Text-fig. A). The vacuole persists through the primary division of the nucleus, and may persist or may begin to disappear in the resting and division phases of the daughter nuclei (figs. 83, 85, 87, 143), until nuclear division in the four-nucleated stage (fig. 89), when it finally disappears, as has been noted by all workers on this subject.

Further, the vacuolated cysts invariably present a well-defined membrane, even at the earliest stage (Text-fig. A), while the appearance of the membrane in other types is quite variable. The membrane may also be well defined and double contoured in optical section in the preliminary stages of nuclear division (figs. 77, 79). But since the vacuole is not found before the primary stages of division of the nucleus, and is associated always with a well-defined membrane, it may be inferred that the formation of such a membrane at this stage of encystment has to do with the appearance of the vacuole. However, since such a membrane may form in the uninucleated cysts without the appearance of the vacuole (figs. 77, 79), the formation of the membrane cannot be said to be dependent on the formation of the vacuole, but rather that in certain cysts the early appearance of a thick and well-defined membrane is related in some manner to vacuole formation, and this formation occurs at the time of primary nuclear division.

The true cyst vacuole must not be confused with the smaller cytoplasmic vacuoles that sometimes persist after the beginning of cyst formation (figs. 77, 78, 154). Although after the daughter nuclei are formed the vacuole sometimes begins to lose size and definite shape (figs. 83, 85, 86), in the uninucleated stage (Text-fig. A), and often in the two-nucleated stage (figs. 87, 143), it is sharply defined and very distinct, and occupies most of the cyst. It probably contains a fluid of some kind, since it shows very delicate gradations of staining (Text-fig. A, figs. 87, 143).

I am of the opinion that the vacuole appears very suddenly, since I could not trace any steps in its development—it was always fully formed when found in the early stages of primary nuclear division. I do not consider the cytoplasmic structure seen in figs. 77, 78, 154, as indicative of vacuole formation, but rather as later phases of fig. 76.

2. *The cyst membrane.* This is variable in appearance and in structure. In its earliest stages it cannot be differentiated from the margin of the ectoplasm. It first appears, in both fresh and stained specimens, as a delicate line.

It may early increase in thickness (figs. 77, 79), or it may be only delicately formed at any stage (figs. 80, 88, 90). When it is well defined the cyst is circular (figs. 77, 91), but when it is delicate the cyst may be oval (fig. 84) or irregularly shaped (figs. 94, 156). External conditions tend to thicken it, for in preparations from moist chambers most of the cysts in the end stage show a very well-defined single or double-contoured membrane. This double contour is frequently referred to as due to the thickness of the cyst wall, but in my opinion it is more probably due to a retraction or a condensation of the cytoplasm (figs. 91, 92). When the retraction is slight and uniform, the appearance is that of a double-contoured membrane, with inside and outside margins (figs. 77, 79), but where the retraction is irregular, the membrane appears single contoured (figs. 91, 92, 155).

3. *Nuclear division within the cyst.* Except in the vacuolated cysts, the beginning of process is not often seen. Fig. 79 shows beginning division of the karyosome, which is said to precede nuclear division, and the nuclei in figs. 77 and 78 are evidently in some stage of division. I have not observed in cysts without vacuoles any definite spindle or oval shaped division of the primary nucleus, although such shapes are encountered during and after the

two-nucleated stage in non-vacuolated cysts. Hartmann and Whitmore did not find primary nuclear division except in the vacuolated cysts, but Werner (1911) gives pictures that represent nuclear division in the cysts without vacuoles. However, the latter's representation of this division, as shown in his figs. 21 and 22, does not agree at all with what I have observed in the vacuolated cysts, and in the later stages in cysts without vacuoles, nor does it agree with Hartmann and Whitmore's presentation. In all the stages of primary division of the cyst nucleus observed by me, the nucleus is elongated, and the chromatin arranged in rows from pole to pole (Text-fig. A), while Werner's figures show:

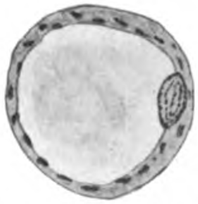


FIG. A.—Uninucleate cyst with vacuole. The nucleus is in mitosis or pro-mitosis. In all the vacuolated cysts with one nucleus, the latter invariably, in my preparations, was in some phase of division.

fig. 21, two solid pear-shaped chromatinic masses connected by a chromatinic filament, within a clear space; and fig. 22, two hemispherical chromatinic masses slightly separated.

Although I found many cysts in various phases of primary division of the nucleus such as is shown in Text-fig. A, I did not see at any time what might be called extrusion of chromidia as described by Schaudinn. Sometimes the spindles were densely coloured, like those shown in fig. 87, and at others the nucleus was very well defined, as in Text-fig. A. But whether heavily or lightly stained, the nucleus was always fully individualised, as noted by Hartmann and Whitmore.

I am at a loss to account for the fact that although practically all phases of nuclear multiplication after primary division of the cyst nucleus could be followed equally well in both vacuolated and non-vacuolated cysts, only in the former was definite primary nuclear division observed. This also appears to have been the experience of Hartmann and Whitmore, since they state definitely that they observed only the beginning of the first nuclear division within the cyst, which is shown in their fig. 10. This figure represents a vacuolated cyst with a large, oval nucleus.

This fact would certainly suggest that the formation of the vacuole is a requisite of primary nuclear division, but it would not explain, if such be true, why the vacuole in some instances vanishes immediately afterwards, as in fig. 82, which shows two resting nuclei in a non-vacuolated cyst, and in others persists to the four-nucleated stage (fig. 89), nor why subsequent nuclear division is the same in vacuolated as in non-vacuolated cysts. Nor would the difference in cytoplasmic structure between the one-nucleated cysts with vacuoles and the two-nucleated cysts without them be accounted for. Probably further research will show definite phases of primary nuclear division in non-vacuolated cysts. According to Schaudinn, the vacuole is formed after primary division of the nucleus, but in this he was clearly in error, as pointed out by Werner, and by Hartmann and Whitmore, whose objections I can fully confirm.

The two-nucleated stage is found equally in cysts without vacuoles and in those with them, as noted by Hartmann and Whitmore. Figs. 80 and 84 show the resting stage, and fig. 83 beginning division of the karyosome. In Text-fig. B and fig. 85 the various aspects of karyosome division are well shown. Fig. 86 shows two spindle-shaped nuclei well advanced in division. Fig. 143 shows a nuclear structure similar to that in fig. 85, in which the difference in staining between the karyosome and the chromatin, by Mann's stain, is well illustrated. Fig. 87 shows two overstained nuclei, a condition resulting from what Hartmann and Whitmore term an increase in chromidial formation.

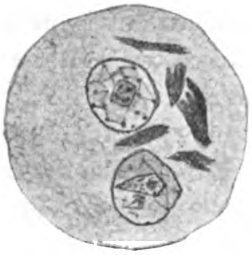


FIG. B.—Two-nucleated cyst, with ' chromidia,' and *areolar* cytoplasm.

Fig. 88 shows the four-nucleated stage prior to division, and Text-fig. C and fig. 89 the four nuclei in division, the former in a non-vacuolated and the latter in a vacuolated cyst.

For the most part, in the two-nucleated stage the nuclei divide synchronously, but sometimes division is delayed in one nucleus while the other divides, forming a three-nucleated stage. Hartmann and Whitmore picture such a stage in both vacuolated and non-vacuolated cysts, and I have seen it in both myself, but not often.

After the four-nucleated stage many of the nuclei divide at irregular intervals, so that five- (fig. 156), six- (Text-fig. D), and seven- (fig. 91) nucleated stages are observed. In the large cysts with the uniformly alveolar cytoplasm at all stages there is sometimes a further division in the eight-nucleated stage, and sixteen nuclei are formed.

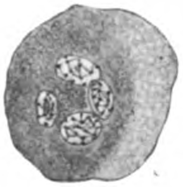


FIG. C.—Four-nucleated cyst, with close meshed cytoplasm in one part, and open meshed cytoplasm in the other. The nuclei are in division.

The mechanism of nuclear division becomes simpler as the development of the cyst advances. In the early stages of primary nuclear division, well-marked spindles, with the chromatin in longitudinal rows, are found. In the daughter nuclei the spindles are not so large, and often the nuclei are oval or round, with well-defined karyosome changes (figs. 85, 143 and Text.-fig. B). In these early phases Hartmann and Whitmore note that in the vacuolated cysts there is a heavier formation of chromatin within the nucleus, and some is extruded as chromidia in the cytoplasm, so that the margins of the nuclei are obscured. This chromatin and chromidia formation, they state, increases progressively from the one to the four-nucleated forms. The chromatin or something else that takes the stain does not appear to be more in evidence in many of the vacuolated cysts, it is true, although in many of these the nuclei are very well defined (figs. 85, 86, 143). I do not agree with Hartmann and Whitmore that the heavily-stained blocks in the cytoplasm of the vacuolated cysts (Text-fig. A, fig. 143) are of nuclear origin, but I hold, as will be explained in Part III, that these so-called chromidia arise from the cytoplasm. Some of the vacuolated cysts do not show the chromidia (?) formation at all (figs. 86, 87, 89).

After the four-nucleated stage division appears to be more primitive. The chromatin is much scantier, and is irregularly arranged in blocks connected by fibrils of lighter-staining substance (Text-figs. C and D), and the karyosome shows only in the resting nuclei (fig. 156), although sometimes a spindle with longitudinal rows of chromatin is found (fig. 91).

The nuclei resulting from division are sometimes of unequal size (Text-fig. D), and this appearance, as well as the occurrence of normal and degenerating nuclei in the same cyst, as pictured by Hartmann and Whitmore, gives rise to a morphology suggestive of reduction division. But a very careful study of all my specimens, over a period of several months, only serves to confirm Hartmann and Whitmore's statement that cyst development follows through repeated nuclear division without autogamy. Fig. 82 might be taken for one of the initial stages described by Schaudinn, that of complete metamorphosis of the daughter nuclei to chromidia, prior to regeneration, but fig. 81 shows the same structure with a very well-defined nucleus partly hidden by the deeply-staining mass. In fig. 82 the nucleus is entirely hidden.

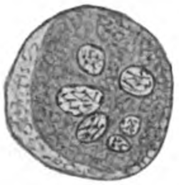


FIG. D.—Six-nucleated cyst. Note the irregular size of four of the nuclei. The two large nuclei are in division. This may be a phase in encystment with more than eight nuclei.

As for the disappearance and reappearance of the nuclei in living cysts, commonly interpreted as indicating reduction and division, I agree with Hartmann and Whitmore that such observations are unsatisfactory. Some fresh preparations will show many cysts without visible nuclei, but fixing and staining will bring out the nuclei, as noted by Craig (1911), and even vital staining will show them. It is more probable that when nuclei disappear and reappear, as I have myself seen them do in the living cyst, division is taking place, and half of the nuclei are invisible. The well-defined spindles in the primary nuclear division are very seldom seen in the living cyst, and then only as delicate ovals without any distinguishing structure.

Exceptionally forms like figs. 70-72 are encountered, which suggest chromidia formation and reduction of the nucleus, and sometimes the nucleus cannot be found, as in Werner's fig. 24*a*. I hold, however, that the correct interpretation of the function of encystment rests upon what is observed in the developmental cycle of by far the greater part of the cysts, and this function is to produce an eight-nucleated resistant organism by repeated division of the nucleus and the formation of the cyst membrane. Why, to produce such a form, so complex a process as the autogamy described by Schaudinn, a process as complicated as the changes following fertilisation in a metazoan cell, should be necessary, I cannot understand. *Entamoeba coli* is a very primitive organism, and as such no doubt will be found to have an equally primitive method of 'rejuvenation' at the time this is needed, when its complete life cycle will have been worked out.

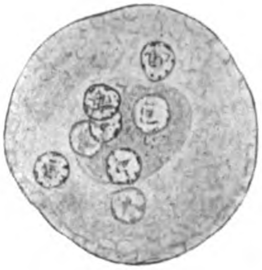


FIG. E.—Eight-nucleated cyst, with central granular and peripheral areolar cytoplasm.

In his very interesting contribution to the study of *Entamoeba blattae*, Mercier (1910) describes in detail a mechanism of encystment differing somewhat from that generally accepted for the *Entamoebae*. One finds in the literature on this subject a belief that nuclear division within the cysts takes place only after the vegetative forms have passed definitely into the encysted stage, as shown by the altered cytoplasm, loss of movement, and formation of the cyst membrane.

In *E. blattae*, however, nuclear division takes place while the organism yet possesses motility and before the membrane is formed, but after extrusion of food particles from the cytoplasm. Mercier states that when the entamoebae have arrived at the end of the vegetative phase they become reduced in size. The nucleus preserves unaltered its characteristic structure, the cytoplasm is still alveolar, but it is free from inclusions and digestive vacuoles. Such entamoebae are now ready to enter upon encystment. In a previous communication Mercier (1909) called these entamoebae 'gamonts.' These 'gamonts' pass from the one- to the eight-nucleated stage before the other phenomena of encystment appear, i.e. separation of the cytoplasm into the granular and the alveolar zones and the formation of the membrane. But these latter phenomena do not follow in ordered sequence, they may appear earlier or later than the eight-nucleated stage, so that what appear to be multinucleated vegetative organisms, but in reality are certain phases of encystment, are not infrequently encountered. Until the membrane is definitely formed, these multinucleated entamoebae possess motility, and were regarded by students of *E. blattae* prior to Mercier as indicating schizogony of the vegetative organisms. Mercier (p. 161) states, 'The fact that encystment can be delayed explains the presence of "gamontes libres" enclosing up to sixteen nuclei.' Mercier's fig. 55, while presenting a cytoplasmic structure finely granular in the centre and alveolar at the periphery, from its shape could readily be interpreted as a multinucleated (16) vegetative organism.

It has been shown that the phenomena of encystment in *E. coli* do not follow in ordered sequence. Also, that certain of the cysts show irregular shapes, and a uniformly alveolar cytoplasm throughout all phases of encystment, with a poorly-defined nuclear membrane in the early stages of nuclear division (Text-fig. B, figs. 80, 84, 156). In such cysts the nuclei are very well formed, and in the resting stages bear a very close resemblance to those of the vegetative organisms. In these cysts only the cytoplasmic structure and the freedom from vacuoles serve to differentiate the cysts from the vegetative forms (figs. 80, 84). However, vacuoles may remain after definite cyst formation has set in (figs. 77, 78, 154). The number of these large cysts in my specimens was, as noted, too small to permit of definite statements as to their complete developmental cycle. But if, in such cysts, division of the nuclei should precede the entire transformation of the cytoplasm from the vegetative to cystic structure, the organisms would then appear as multinucleated entamoebae of the vegetative stage, especially with regard to the structure of the nuclei.

The nuclei of the three-, four- and six-nucleated organisms pictured by Hartmann and Whitmore in their figs. 6, 5, and 7 as vegetative entamoebae do not show morphological value for differentiation from cysts, for equally as well formed and very similar nuclei are found in undoubted cysts, and indeed are shown by the authors in their figs. 21 and 22, which represent four-nucleated cysts.

There remains for consideration the cytoplasmic structure of the multinucleated vegetative organisms of Hartmann and Whitmore. This is undoubtedly of the vegetative type, showing as it does vacuoles and inclusions. But if the metamorphosis of the cytoplasm from the vegetative to the cystic type were delayed until after nuclear division were well advanced, it is readily possible to conceive of Hartmann and Whitmore's multinuclear vegetative forms as representing such a stage, and their fig. 26, which represents the eight-nucleated stage in one of the large cysts, as a further phase in the development of their fig. 7, the six-nucleated vegetative form.

In considering the above suggestions, it should be remembered that, as compared with the vast amount of work on some of the other species of Protozoa, very little research has been done on *E. coli*, especially with accurate staining methods. Hartmann and Whitmore themselves do not show a complete schizogony, nor could I, after several months' search through many preparations, find all phases of development in the large alveolar cysts. In fact, I found very few such phases, and have represented all of them in figs. 80, 84, 93, 94, 156, and Text-fig. B. I believe fig. 154 is the initial stage of one of these cysts, and it is not difficult to understand that should the nucleus in a cyst of this kind divide before the cytoplasm undergoes further alteration, how readily a so-called vegetative form might be produced.

Especially in the study of the *Entamoebae* of man, previous work of high authorities has been accepted without question, only to be later discarded, as is shown by Craig (1913) in his latest communication. In my opinion, as previously noted, there is very little evidence upon which to base an affirmation of eight-nuclear schizogony, and the irregularity with which the phenomena of encystment often occur has received scanty, if any, attention from previous workers.

ENTAMOEBAE RESEMBLING *E. COLI*, BUT CLASSIFIED AS SEPARATE SPECIES

A careful study of the contributions of v. Prowazek on *E. williamsi* (1911), *E. poleki* (1912*a*), *E. hartmanni* (1912*b*), and *E. bütschlii* (1912*b*) has convinced me that this writer worked with very insufficient material, and for the most part had to do with various phases of *E. coli*, as Hartmann and Whitmore note, and very probably, in one instance, with a mixed infection.

v. Prowazek states that *E. williamsi* was found associated with *E. coli* and several other intestinal inhabitants, but was differentiated by reason of the presence of excretion crystals in the cytoplasm, the method of movement, the kind of food taken up, the particular chromidia formation, the appearance of ten-nucleated cysts, and various details of development.

Crystals, when these are present in the faeces, are very frequently taken up by *E. coli*, and the author does not state whether he observed these crystals in the faeces. The method described for the formation of pseudopodia in *E. williamsi* is seen also in sluggish *E. coli*. The latter will phagocyte practically any material free in the stools, as I have noted. The so-called chromidia, either in blocks or in crystal-like masses, as represented in the author's drawings, are often found in *coli* cysts, as shown in my fig. 90, Text-fig. B. The ten-nucleated cysts would not be characteristic, unless they were clearly shown to be the end stage.

In a later communication v. Prowazek (1912*a*) states that he looks upon *E. williamsi* as a variety of *E. coli*, but it seems to me that most of his drawings represent what is commonly enough seen in infections with the latter organism.

Nor can I find in v. Prowazek's descriptions of the other entamoebae any reason to believe that new species have been demonstrated. *E. hartmanni* was found in one case only, with scanty *coli* forms present also. The pictures given for *E. hartmanni* resemble very closely Elmassian's for *E. minuta*, and what I find in the encystment of the pathogenic species (fig. 10) showing the eight-nucleated stage of *E. hartmanni*, although measuring only 11·5 μ, cannot be differentiated from a *coli* cyst, or perhaps a *tetragena* cyst in which multiplication has passed beyond the four-nucleated stage, as described by Whitmore (1913), and also observed, but only twice, by myself.

The woman from whose stools the preparations were made was not suffering from dysentery, which is good reason to believe that in this instance v. Prowazek had to do with a latent stage of the pathogenic species, as described previously in this article. The author remarks that the eight-nucleated stage of *E. hartmanni* was very seldom found, but has also stated that the infection was associated with 'spärlichen Coliformen.'

v. Prowazek found *E. bütschlii* only once, in a child who was infected also with *Ankylostoma*, *Ascaris*, *Trichocephalus*, *Trichomonas*, and what is more important, *E. coli* likewise. The nucleus pictured in his fig. 13 is intermediate in type between that of *tetragena* and that of *coli*. However, as pointed out elsewhere, I regard nuclear morphology *alone* as a very unsafe criterion for classification of species. His figs. 14 and 15, which represent two- and three-nucleated vegetative entamoebae, in my opinion represent equally as well enucleate entamoebae that have phagocyted some organism, probably a yeast. The cysts of *E. bütschlii* can in no way be differentiated from those of *E. coli*.

E. poleki was found once only. It is very difficult from the illustrations presented to say whether entamoebae or something else are represented.

Koidzumi (1909) states that the cytoplasmic structure of *E. nipponica* resembles very closely that of *E. tetragena*. As pictured by the author, the typical nuclear structure is identical with that of degenerating *tetragena* forms and some varieties of the *coli* nucleus, as Hartmann and Whitmore point out.

I could not find sufficient information as to *E. tropicalis* and *E. undulans* to compare these organisms with my own findings.

PART III

CLASSIFICATION OF THE *ENTAMOEBAE* OF MAN FOUND IN THE CANAL ZONE

If, in seeking to classify the *Entamoebae* observed in the course of my study I were to arrange them with respect to variations from a restricted and definite morphology, I could affirm the existence not only of two or three species, but of several more. I have been led to the belief, however, as the result of my work, that neither morphology alone nor variations in the cycle of development afford a sound basis for classification. It is an accepted fact that the Protozoa vary in structure and in development in proportion to alterations in the media in which they live, and I can see no reason to believe that the *Entamoebae* of man should prove to be exceptions to this fact.

In confirmation of Walker's (1911) opinion, I can state that two cycles of development are found, the one ending in four-nucleated, the other, in eight-nucleated cysts. The entamoebae whose cycle ends in four-nucleated cysts may at times become pathogenic, but there is considerable evidence to show that a portion of their cycle, at least, is not accompanied by symptoms of dysentery. On the other hand, that phase of their existence in which they are known as the 'large vegetative forms' is always associated with ulceration, and very frequently with dysentery. As far as my own research is of value, I can confirm the opinions of those who state that the entamoebae whose cycle ends in the eight-nucleated cysts are without ill effects on their hosts.

In each cycle variations from the more common types are met with, and these variations are more marked in the entamoebae that produce four-nucleated cysts than in those that produce eight-nucleated cysts.

Following Walker, then, I divide the *Entamoebae* of man into two groups, on the basis of the development of their respective cycles:—

Group 1. Entamoebae whose end stage in the human host is a four-nucleated cyst.

Group 2. Entamoebae whose end stage in the human host is an eight-nucleated cyst. (See Part II, *E. coli*).

GROUP I

The organisms of this group include those described in Part II as (*a*) entamoebae associated with acute dysentery, and (*b*) entamoebae associated with mild, or latent dysentery, and sometimes present without either history or symptoms of dysentery.

It will be remembered, however, that Schaudinn gave for *E. histolytica* a cycle that differs markedly from any of those described in Part II for the various types. The various explanations given by authors who have failed to confirm Schaudinn's observation as to development do not appear to me to be altogether satisfactory, as will be shown. Many observers have not seen the forms described by Schaudinn, and are inclined to dismiss his observations altogether, while those who have described forms similar to the end stages described by Schaudinn account for his deductions as the result of erroneous interpretation.

According to Schaudinn, *E. histolytica* reproduces itself in the vegetative stage by simple division, and by a kind of schizogony in which the parent organism, after the nucleus has been distributed to the cytoplasm, divides into a number of small entamoebae. The end stage of the cycle is an organism about 20 μ in diameter, whose nucleus is also distributed to the cytoplasm. Later, the peripheral part of the entamoeba is pinched off in small buds, each bud containing a portion of the distributed nucleus, and surrounded still later by a membrane. Various phases of the distribution of the chromatin to the cytoplasm are described, as is the formation of chromidia prior to budding.

These processes, reproduction by schizogony, or gemmation, and spore formation by budding, were never satisfactorily confirmed as to function, although identical morphological appearances have been seen and pictured by Craig (1905-08-11), Werner (1908), and Hartmann (1909), as noted in Part I. Many others have sought for them in vain, and Hartmann's confirmation was largely based on Schaudinn's preparations.

Except for Schaudinn's statement that spore formation by budding takes place only during healing, while reproduction by schizogony, or gemmation, takes place during the height of the dysentery, the two processes are almost identical, being differentiated only by the formation of buds in the one, and of young entamoebae in the other.

After a year's fruitless research for anything that corresponded to budding, although I encountered the preliminary stages of nuclear dissolution (figs. 5-9), I read the accounts of Viereck (1907) and of Noc (1909) which I have abstracted in Part I. Each of these authors, in referring to the method by which Schaudinn obtained his specimens of gemmation and spore-formation, state distinctly that entamoebae showing these phases were found in preparations *watched for some time under the microscope.* I had, as noted, followed organisms of the *histolytica* type as far as the alteration of the nucleus and the beginning of chromidia formation (figs. 5-9), but had found no further development.

Acting on the information in the accounts of Viereck and Noc, I took preparations of the organisms of the *histolytica* type in the vegetative stage, and placed them between slide and coverglass for periods of from fifteen minutes to several hours, and also placed them in a moist chamber for corresponding periods. The results, whether in living organisms, or in those wet-fixed and stained, were the same. Although at the time when the stool was passed only a very few of the organisms contained chromidia, and the nuclei were nearly all normal, within thirty minutes to two hours the changes shown in figs. 10-16 took place. Not only did certain of the organisms show what might be regarded as the preliminary stages of reproduction by gemmation (figs. 12-14), but others showed what might be interpreted as the preliminary stages of spore formation (figs. 15-16). My figures 12-16 correspond very closely to those drawn by Hartmann from Schaudinn's preparations, and also with those drawn by Werner for these phases.

Later, by the same methods, I found similar appearances in the large vegetative organisms at all phases of their cycle, and even in the pre-cysting generation.

I regard these changes as purely degenerative, and for this reason I am forced to believe that, in so far as reproduction by gemmation, and spore formation by budding are concerned, Schaudinn mistook degeneration for development, not only in those entamoebae which in the pre-cystic generation show nuclear alteration and chromidia formation, but also in the large vegetative forms when these were left between slide and coverglass. From the moment that the stool is passed, even under the most favourable environment, the vegetative entamoebae rapidly degenerate, and no deductions as to schizogony or spore formation can be made from preparations which are not fixed immediately.

Figs. 5-9, which form a part of Schaudinn's cycles of schizogony and of spore formation, occur plentifully in the pre-cystic and cyst-forming stages, but figs. 12-16, from moist chamber preparations, are very infrequently encountered in man in the freshly-passed stool, although there are many of them in moist chamber preparations.

Whitmore (1913) believes, from Hartmann's (1912) account, that Schaudinn described the disputed phases in *histolytica* from degenerating entamoebae in the pre-cystic and cyst-forming generations. In so far as the preliminary stages are concerned, I can agree with this, as noted above, since figs. 5-9 represent the transition period between the stage of the large vegetative organisms and the pre-cystic generation. But the later stages, figs. 10-14, as I have stated, are found very rarely in human stools. Figs. 12-14, which represent the stage described by Schaudinn as complete transformation of the nuclear chromatin to chromidia, prior to budding, I have found *only* in moist chamber preparations, and in those left for some time between the slide and coverglass.

It would be of the greatest interest if those who are in a position to give the information would state definitely whether Schaudinn found the disputed phases in freshly-passed stools, or whether he found them only in those preparations which he watched for some time under the microscope.

Darling (1913*c*) found similar forms in the fourth remove during the passage of a strain of the *tetragena* type through cats, and regards them as degenerative.

Craig (1913) has come to the conclusion that the forms described by him as confirming Schaudinn's description of the cycle of *histolytica* were degenerating organisms, which he found for the most part in the stools of patients under treatment by irrigations.

Although the sum of the evidence presented above justifies the belief that Schaudinn was in error in his interpretation of the development cycle of *histolytica*, there remains for explanation his experiment as to the transmission of amoebic dysentery. This experiment, as translated by Craig (1911, pp. 163-165) was as follows:—

> 'From this case I (Schaudinn) took a small quantity of faeces, divided it into three parts, dried each in the air, and mixed it with sufficient water for about twenty crush preparations under coverglasses. These preparations were carefully examined, the examination being conducted with a mechanical stage, and requiring many hours. No forms resembling the cysts of *Entamoeba coli* were found, but the small spores of *Entamoeba histolytica* were noticed in large numbers, but no vegetative organisms could be demonstrated. The coverglasses were then removed, the faeces washed in distilled water, and ten such preparations were mixed with enough distilled water to form 1 c.c. of the mixture. The faeces of the animal to be experimented upon, a healthy, strong, young cat, were carefully examined for amoebae, and none could be demonstrated. To this cat I gave the 1 c.c. preparation mentioned above, mixing it with milk. On the evening of the third day the cat passed bloody mucoid faeces, and an examination showed the presence of great numbers of typical *Entamoeba histolytica*. In the afternoon of the fourth day the cat perished. Dissection showed typical ulcerous dysentery of the large intestine, and the immigration of the amoebae into the epithelium could be easily established.
>
> 'I will mention yet another experiment which goes to prove that the permanent spores by themselves are capable of producing a new infection. The faeces of the cats developing dysentery contained only the vegetative stages of the amoebae, no spores being found. When large quantities of the faeces were given to a cat it remained well, and for four weeks showed no amoebae in its faeces. It was then fed with the remnant of the dried faeces used in the first experiment, which contained multitudes of spores, and after six days the amoebae began to appear in the faeces. Being older and larger than the other cat, it proved more resistant to the infection, and did not die until two weeks later. The autopsy showed the lesions of typical amoebic dysentery.'

According to Darling (1913*a*), whereas after three to six days in a moist chamber the cysts all germinate, dried material loses its infectiousness as soon as desiccation is completed, although the cysts remain in the original quantity, but altered and warped in appearance. I can confirm these experiments, and may add that, like Dr. Darling, I was also unsuccessful in infecting cats with dried material, although Dr. Darling had but little difficulty in producing amoebic dysentery in cats from fresh material, collected by himself, or supplied by me, when the material contained cysts with four nuclei. Some of my material was dried and preserved for six weeks, and although the dried cysts could be made out, I could not infect kittens, but the same material freshly used, or kept moist for one or two days, in Dr. Darling's experiments resulted in infecting kittens.

In view of these data it is very difficult to explain Schaudinn's success in infecting kittens with the dried spores of *histolytica*. If cysts had been present in any number in his material, it is hardly probable that he could have overlooked them. But even had cysts been present, the air-dried material would not have been infective, unless it is shown later that it is possible to infect with such material. The 'spores' which Schaudinn thought to be the infective agents have been proved to bear no relation whatever to the entamoebae, whatever else they may have been.

To the best of my knowledge, this experiment of Schaudinn's was never confirmed, yet for many years it was accepted as absolute proof of the reproduction of *histolytica* by spore-formation, so great a weight had his name in Protozoology.

The type of vegetative entamoeba described as *histolytica* by Schaudinn has not escaped criticism as to its validity. As was shown in Part I, this type has been recognised by nearly all workers on the *Entamoebae*. Some of these workers described infections in which variations were encountered (see particularly the abstract of Viereck's article in Part I), while others insisted upon what may be called 'single' infections with the type. In Manila, Walker (1911) found the *histolytica* type more common than the *tetragena*, but stated that the latter was always associated with the former, while transitional forms were common. Craig (1905-08-11-13) emphasised the validity of the type, stating that it did not go on to cyst formation, neither did it develop into the *tetragena* type. This has been my own experience, as previously reported (1912*b*).

On the other hand, Hartmann (1912) states emphatically that in working over Schaudinn's material all of the infections, except one that the latter had described for *histolytica*, proved to be *tetragena*, and also that all of the entamoebae in the preparations brought by Whitmore from Manila were *tetragena*, not *histolytica*. *Tetragena* only was found in all of Hartmann's other material, collected from different sources. 'I can, therefore,' he states, 'at present assert with great surety . . . . that almost all cases of amoebic dysentery are not brought about by *E. histolytica*, but by *E. tetragena*.' (See Part I, abstract of Hartmann's (1912) communication).

Whitmore (1913) also is sceptical of the validity of the *histolytica* type. 'I have had opportunity,' he writes, 'to study preparations from four cases in which the entamoebae were of the *histolytica* type, and in every case, either in the original preparations, or by re-straining, I have been able to bring out the *tetragena* type of nucleus in a good proportion of the amoebae. . .

'But it seems certain that the *histolytica* type of nucleus is commonly met with in the large vegetative amoebae in active dysentery, and this is to be expected . . . . The nuclear membrane is not a permanent structure, but its presence or absence depends mainly on the amount of achromatin contained in the nucleus. The cyclic change, described for the *tetragena* type of nucleus, very often leaves the karyosome as but little more than a central dot; and the amount of chromatin in a nucleus varies under conditions of age and vegetative function. . . .

'Then, even in acute dysentery, undoubtedly many of the amoebae are degenerating, and this would add to the number of *histolytica* forms. . . .

'On making a re-study of a large number of preparations, with the special object of finding the *histolytica* type of nucleus, I have been very strongly impressed with the comparative rarity with which I find a large vegetative amoeba without distinct indication of the cyclic change which Hartmann described as so characteristic for the *tetragena* type of nucleus.'

Notwithstanding the criticism of Hartmann and of Whitmore, I believe in the validity of the *histolytica* type of entamoeba, and base my reasons for so doing not only on the nuclear structure, but as well on certain facts in the behaviour of this type in the human host. The entamoeba described by me in Part II as Type A has peculiarities that seem to me to justify its classification as a distinct type, but not, as I shall point out, as a species.

It is certainly true that *histolytica* forms occur in infections in which most of the entamoebae show the *tetragena* type of nucleus, and the converse is also true. I found this early in my work, and noted it (1912*b*). But I have now to do with infections in which by far the greater number of the entamoebae are true to type. The objection raised by Hartmann, that he found on working over Schaudinn's preparations most of the entamoebae were of the *tetragena* type does not invalidate the existence of infections in which most of the entamoebae are of the *histolytica* type. Whether or not the *histolytica* type occurred in Schaudinn's preparations, whether or not Schaudinn had in mind the entamoeba described by me as Type A, or whether or not he described for *histolytica* degenerating forms of *tetragena*, does not alter the fact that I have found here in the Canal Zone infections in which most of the entamoebae show the *histolytica* type of nucleus. Such infections are marked off from those in which most of the entamoebae show the *tetragena* type of nucleus not only by the morphology of the nucleus, but also by the clinical course, as I have pointed out. Even if it be true that Schaudinn described *histolytica* from degenerating forms of *tetragena*, the findings of Craig, Walker, and myself demonstrate that a type of vegetative entamoeba exists, identical with that described by Schaudinn for the vegetative stage of *histolytica*, and is frequently encountered in acute dysentery, especially of primary origin. I have no means of knowing if Schaudinn saw entamoebae of my Type A, but whether or not he saw them, they are present in the Canal Zone and elsewhere as an individual type.

The degenerating entamoebae found in the pre-cystic and cyst-forming generations of the *tetragena* type have nothing in common with the *histolytica* type, as I understand the latter. Figs. 1, 2, and 3 show *histolytica* in acute dysentery; figs. 33 and 34, degenerating entamoebae from *tetragena* infections. In the former the nuclei are true to type, and the cytoplasm is normal; while in the latter there is a very wide variation in nuclear appearance, and the cytoplasm, after any method of fixing and staining, shows unmistakable signs of degeneration. Especially is this true after dry fixation and staining with Hastings and Giemsa. In preparations from acute dysentery nearly all of the entamoebae will show normal cytoplasm (figs. 124-126), while in preparations from the pre-cystic and cyst-forming generations there are not infrequently more degenerating than normal forms (figs. 130-131).

I have not seen any of Schaudinn's original preparations, nor any of Hartmann's, that show the degenerating forms, but the description given by Hartmann (see Part I, abstract of Hartmann, 1912) of such forms does not in any essential agree with mine for the *histolytica* type.

I can agree with Whitmore's statement that at certain phases of the cycle the karyosome is but little more than a central dot, thus giving a nuclear picture similar to that of *histolytica*. But Type A infections showing nuclei of the *histolytica* type persist unaltered in most instances over several days, and sometimes even for several weeks, whereas in the *tetragena* type not only do many of the entamoebae show very well defined karyosomes at all times, but also when many of the nuclei are in the condition described by Whitmore as resembling *histolytica*. Later, the well-defined karyosomes will again appear, which is not true in *histolytica* infections.

Whitmore (1913), as noted, states that he rarely found large vegetative entamoebae that did not show distinct indications of the cyclic changes in the karyosomes. Precisely how much indication of these cyclic changes is necessary to classify entamoebae as of the *tetragena* type depends to some extent on the personal equation, and also, as Whitmore points out, on the technique employed. But when due allowance has been made for variations in interpretation and in fixing and staining processes, I find that in my study it has been possible to demonstrate two series of cases: in the one the *histolytica* type of nucleus predominates, and in the other the *tetragena* type.

In a comparison of my own preparations of *tetragena* nuclei and those lent to me by others, with my own preparations of *histolytica* nuclei, I find that the structure of the karyosome, while variable, distinctly sets apart the former type from the latter. I may note here that only in my own preparations from cases of acute dysentery have I found 'single' *histolytica* infections. In all of the preparations sent me from abroad the *tetragena* type of nucleus could plainly be recognised. One might think that this is because of differences in technique, but since I obtained my own *tetragena* preparations with the same reagents and under the same conditions as I obtained my *histolytica* preparations, and there was also no essential difference in morphology between my own *tetragena* preparations and those sent me by others for comparison, I do not think that my finding of 'single' *histolytica* infections can be charged to faulty technique.

Clinically, Type A, or the *histolytica* type, is found in acute primary amoebic dysentery, and in relapses of exceptional severity. It is not found as a type (although, as noted, individuals of the type may be encountered) in chronic dysentery, especially when there are alternate periods of dysentery and constipation. It has been my experience that the more the entamoebae conformed to the *histolytica* type, the severer was the dysentery. Even in the periods of apparent remission, when the acuteness of the dysenteric symptoms had somewhat subsided, when the entamoebae show alterations in the nuclei and chromidia are formed (figs. 6-9), there is not a conversion later to the *tetragena* type of nucleus (figs. 22-25*b*).

I did not find that Type A infections went on to cyst formation. I could not, however, observe such infections for longer than three weeks. But within three weeks, and very frequently within a week or ten days, encystment invariably followed in my *tetragena*, or Type B, infections.

I consider, then, as distinctive of infections of the *histolytica* type, (*a*) the characteristic nucleus; (*b*) the clinical course, the disease showing but slight improvement with rest in bed and a restricted diet, and the acuteness of the dysentery; and (*c*) the non-appearance of encystment within the periods of observation at my disposal.

Type B, or the *tetragena* type, as noted, is associated with chronic and sub-acute dysenteries, and with ulcer formation without symptoms of dysentery when the entamoebae are found in the course of the routine stool examinations. Not infrequently, in stools following the exhibition of salts some bloody mucus is found, in which very beautiful specimens of the type can be demonstrated. The histories in such cases are very indefinite, but as a rule a history of acute dysentery is not given. Analogous to these cases in life are those found here by Dr. H. C. Clark, Pathologist to Ancon Hospital, at autopsy. Dr. Clark frequently found all stages of ulcer formation in the caecum or in the appendix at autopsy when there had been no history or symptoms of dysentery while the patient was in the hospital. Sometimes the lesions were quite extensive, and could not be differentiated pathologically from those in which amoebic dysentery was given and accepted as the cause of death. Such cases point unmistakably to a well-defined variation in the virulence of the entamoebae, and this variation appears to be in relation to the relative prevalence of one or the other type of nucleus. In two fatal cases of amoebic dysentery, the entamoebae were of the *histolytica* type.

In all of my Type B infections the cyclical changes in the karyosomes, and the karyosomes themselves, were well defined (figs. 22-25*b*). The dysentery in all of these cases was relatively much milder than in 'single' Type A infections. Without treatment, except rest in bed and a restricted diet, and some few doses of salol, there was a decided improvement in the symptoms, and an apparent cure. The dysentery may be acute at the onset, but it is not as acute as in the Type A infections, and the stools as a rule are not more frequent than six to ten in twenty-four hours. They become watery in two to three days, and within a week longer are formed, and blood and mucus are no longer present. In the watery stools many of the entamoebae lose the characteristic structure of the nucleus (figs. 22-25*b*), and are identical with those found in the watery stools in Type A infections. But in the Type B infections the cycle goes on to completion, as described in Part II. As the stools become semi-solid, most of the nuclei take on again the typical *tetragena* type (figs. 35-40), while others take on the *minuta* type (figs. 26-31). In one of my cases the latter type appeared exclusively prior to cyst formation.

Cyst formation, in all of my cases, followed in the same manner, as noted in Part II. Dr. Elmassian, as stated in Part II, found a variation in the cysts accompanying the vegetative entamoebae (figs. 109-110), as compared with cysts of the true *minuta* type (figs. 111-113).

Characteristic for the Type B infections are : (*a*) the nuclei showing the typical karyosomes, with the cyclical changes; (*b*) the clinical course, ending in an apparent cure, without medication; and (*c*) the forming of four-nucleated cysts in all cases when the cause of admission was amoebic dysentery. Cysts were not always found when occasional vegetative entamoebae were met with in the course of disease other than amoebic dysentery.

Type C (figs. 17-21) is, as noted, morphologically intermediate between Types A and B. Very probably most observers would class the nuclei of this type as *tetragena*, but to me there is not the distinct marking off of the karyosome and the cyclical changes that one finds in true Type B infections. In the development of the cycle, however, there is a difference between Type C and Type B infections. Whereas in the latter for the most part the entamoebae followed the developmental cycle described by Hartmann (1912, see Part I), in the former the organisms passed from the large vegetative forms (figs. 17-21) through the *minuta* type of vegetative organisms (figs. 26-31) to the cyst formation common also to the Type B infections, except that in the pre-cystic generation the nuclei were of the *minuta* (figs. 26-31), not the *tetragena* type (figs. 35-40).

In case 1, entamoebae of the type found in watery stools in both *histolytica* and *tetragena* infections (figs. 5-9), followed the disappearance of the large vegetative forms. Then the cycle passed through the *minuta* type to encystment.

I have not sufficient data on this type of infection to make any generalization as to the accompanying clinical course. The protocols of the three cases are given in Part II. As noted, one of the infections passed in the short time of three days from the stage of the large vegetative organisms to encystment, the shortest cycle of which I could obtain a record, either in the literature or in my own experience.

ENTAMOEBAE ASSOCIATED WITH MILD, OR LATENT DYSENTERY

Depending for the most part on whether the dysentery is subsiding, or whether it is latent (i.e., in the interval between attacks), two types of entamoebae may be recognized under this heading. It is here, also, that the entamoebae producing four-nucleated cysts, when there has been no history of dysentery, or at the most only some vague abdominal distress, should be classified.

(1) Entamoebae found during convalescence in Type B infections.

(2) Entamoebae producing four-nucleated cysts, not associated with a history or with symptoms of acute dysentery, and sometimes found when there has been no history nor symptoms of dysentery.

Entamoebae of Class 1 are identical with those described by Hartmann (1912) for the pre-cystic and cyst-forming generations of *E. tetragena*, and by me for the end phases of the cycle of Type B (figs. 35-40).

In Class 2 there is a well-marked variation from the preceding class. As stated in Part II, entamoebae of this class correspond to the *minuta* type of Elmassian.

The difference between the two classes lies in their nuclear morphology and in the physiology and pathology of the entamoebae. In Class 1 the nuclear morphology, while variable, is distinctly of the *tetragena* type, and many degenerating organisms are encountered, whose nuclei show all stages of disintegration. In Class 2 the vegetative organisms are remarkably constant in morphology, and degenerating entamoebae are infrequently found (figs. 26-31). The general impression gained by a study of Class 1 entamoebae is that of a cycle whose vitality is waning; by a study of Class 2 entamoebae, that of a cycle whose vitality is uniform and constant. But it must not be forgotten that Class 2 entamoebae are found at times in the cycle of the *tetragena* type.

The clinical differences are also well defined. Class I entamoebae belong to the *close* of a cycle associated a short time previously with more or less acute dysentery, and Class 2 entamoebae to a cycle associated with a persisting infection that for some reason has not taken on any marked degree of pathogenicity, or to a latent infection that has not given rise to symptoms of dysentery.

The cyst-forming, or end stage, was the same in all instances in which it was observed. Dr. Elmassian found in some of my preparations associated with large vegetative organisms of the *tetragena* type the cysts shown in figs. 109-110, and these he distinguished from cysts of the *minuta* type by the larger size and difference in nuclear morphology. Except for this, however, the cysts were the same in all of my other preparations, whether obtained after acute dysentery, or whether associated with entamoebae of the *minuta* type. By actual measurements some of the largest cysts were found in typical *minuta* infections, and some of the smallest cysts at the close of a well-defined cycle beginning with the large vegetative forms of the *tetragena* type, and the reverse was also true.

CLASSIFICATION OF GROUP I; ENTAMOEBAE WHOSE END STAGE IN THE HUMAN HOST IS A FOUR-NUCLEATED CYST

In a previous communication, read before the Canal Zone Medical Society in May, 1912 (1912*b*), and published in the New York Medical Journal for April 5th, 1913, in discussing the difference between entamoebae associated with acute dysentery, and those associated with chronic dysentery, I stated:—

> 'These facts raise the interesting query as to whether the "chronic amoebic dysenteries," with their exacerbations and remissions extending over many years, which are reported from all parts of the tropical world, may be due to *Entamoeba tetragena*, while the acute and rapidly fatal forms of amoebic dysentery have as their etiological factor *Entamoeba histolytica*. Or, if one believes with those who hold that there is a single species only of pathogenic entamoebae, perhaps one may state that the variety known as *tetragena* is not as noxious as that known as *histolytica*.'

At that time I based my reasons for accepting *histolytica* and *tetragena* as distinct species on the grounds that in *histolytica* infections I had been unable to demonstrate a complete developmental cycle ending in the production either of four-nucleated cysts, while Schaudinn's 'spore-forming' phase, although not fully confirmed biologically, had not been disproved. I stated also that I did not regard nuclear morphology as a secure basis for differentiation.

Even at the present writing I have not witnessed a complete developmental cycle beginning with the vegetative entamoebae of the *histolytica* type. But the finding in January, 1913, of the entamoebae intermediate in nuclear structure (Type C) between *histolytica* and *tetragena*, passing through a complete developmental cycle ending in four-nucleated cysts, and the demonstration about the same time, of the 'budding' and 'spore-forming' phases of *histolytica* in moist chamber preparations and in preparations left for some time between the coverglass and slide, convinced me that Type C was a connecting link between *histolytica* and *tetragena*, and that sooner or later the complete cycle will be demonstrated. As noted, I could not keep any of my cases under observation longer than three weeks, but I am confident that should opportunity arise whereby infections with pathogenic entamoebae can be followed from the beginning to the end of the cycle, it will be found that all of the forms described by me as ending in four-nucleated cysts will be found at one stage or the other of the infections. Such an opportunity would be presented by artificial infection in man having its beginning in the feeding of material containing four-nucleated cysts, but I have had no opportunity to conduct such an experiment, and do not believe that it will be possible for me to obtain it. Unless I had absolute control over an artificial infection for a period of two or three years, I should not feel justified in bringing it about. There is no more serious infection in the tropics than an amoebic dysentery that has escaped control, and although the bismuth treatment will eliminate the organisms in the intestinal walls, there is no way of controlling an abscess elsewhere, if the abscess is formed prior to the beginning of the treatment. The emetine treatment, although it has given brilliant clinical results, cannot as yet be relied upon as a specific against the formation of an amoebic abscess.

I sent specimens from the three Type C infections to Dr. Chas. F. Craig, stating that in my belief they showed a transition stage between the *histolytica* and *tetragena* types of infection, and in one case what I took to be *histolytica* itself going on to encystment, which at the time I did. Dr. Craig's opinion of this is given in his recent publication (1913). He regarded Type C as resembling more *histolytica* than *tetragena* in the vegetative stage, and in Case 2, which at first I took for a 'single' infection, he demonstrated both *histolytica* and *tetragena*, which I found also to be true on later study. The preparations from these three cases, with those from a similar case of his own under observation at about the same time, were the basis of Dr. Craig's revision of his former belief that *histolytica* and *tetragena* were distinct species. Since the beginning of the controversy as to whether the two species were identical or not, Dr. Craig has always held that a proved differentiation would not be obtained until the entire life cycle had been worked out.

The mistaken belief that the *histolytica* type represents no more than degenerating forms of *tetragena*, while in reality it represents a very well-defined phase of the cycle associated with acute and continued amoebic dysentery, has delayed a proper classification, just as 'the unwarranted emphasis laid by certain authorities upon nuclear structure (Craig, 1913)' for so long a time was productive of the diversity of species.

Craig gives to Walker (1911) the credit for first clearly showing that the two varieties have the same end stage, or in other words, belong to a single species, and as far as I can judge from the literature at my disposal, it is to Walker that this credit belongs.

In order clearly to comprehend the conditions under which the different types of entamoebae are met with, it is necessary to understand as fully as possible the clinical course and pathology of amoebic infection, and to associate with these the life cycle of the entamoebae.

Like other protozoon infections, that with the entamoebae follows a protean course. It may be fatal in a short time; it may be severe for a long or short time; it may become chronic, with alternating periods of dysentery and constipation, or with constant passage of unformed stools; it may be mild, and cause but little discomfort; ulceration of the large bowel, quite extensive, may be present without giving rise to symptoms; and an infection with four-nucleated cysts as the end stage of the cycle may be in force without any indication of ulceration or of dysentery.

The severity of the infection may be apparent in the physical condition of the patient, who is emaciated and greatly distressed by the frequency of the bowel movements. The localization of the ulcers in the rectum may give rise to severe dysenteric symptoms without emaciation, and ulceration may be present in the upper part of the bowel without severe, or even without appreciable clinical symptoms of dysentery, while the general health of the patient is steadily declining.

From these data it is plain that severity of the dysenteric symptoms alone will not always be a guide to the severity of the infection. What appears to be a very severe attack of amoebic dysentery will not infrequently clear up in a few days with rest in bed and a restricted diet, while milder clinical cases, with the same treatment, will persist unchanged for weeks and months. So that it is evident that in the classification of amoebic dysenteries according to symptoms, one must take into account more than the severity of the dysentery itself. The condition of the patient, the onset or delay of amelioration of the symptoms, and the intervals between exacerbations, must all be taken into account.

It has been my experience here that a general classification of the forms of amoebic dysentery may be had by correlating with them the types of entamoebae encountered. A precise classification can be obtained or disproved only by a study of far more cases (and over a much longer time) than have been at my disposal.

RELATION OF DYSENTERIES TO THE TYPES OF ENTAMOEBAE ENCOUNTERED

Class I. *Acute amoebic dysentery, proving fatal, or persisting unimproved as long as treatment is withheld.* In this class the entamoebae are of the *histolytica* type, or Type A. I have not observed cases in this class longer than three weeks. In them, the entamoebae, while not going on to encystment, at times took on the appearances shown in figs. 6-9, which appearances were found at times prior to the pre-cystic generation in Types B and C also.

Class 2. *Amoebic dysentery which, while more or less severe at the onset, persists for a few days only and subsides with rest in bed and a restricted diet.* The entamoebae showed changes corresponding to the alterations in the stools, and were of the

Type B, or *tetragena* type. During the acute stages only the large vegetative organisms were found, and as improvement took place the entamoebae passed through the pre-cystic and cyst-forming generations until the stools were formed, when the four-nucleated cysts were found. In one case there was a passage of the cycle through the *minuta* vegetative stage, *tetragena* organisms reappearing in the cyst-forming stage. All five cases in which the complete cycle was followed gave histories of attacks of dysentery prior to that on admission.

Class 3. *Primary amoebic dysentery, in which a cycle from the large vegetative forms to encystment was followed.* The histories of the three cases in this class, Type C infections, were given in Part II. Except that the dysenteries were primary, the clinical course was much the same as in Type B infections. Characteristic especially for this class was the absence of the *tetragena* nucleus in the pre-cystic and cyst-forming generations, the entamoebae taking on in these phases the *minuta* type of nucleus.

Class 4. *Mild, or latent dysentery.* In those cases in which there had been a history of recent acute dysentery, the entamoebae corresponded to the pre-cystic and cyst-forming generations of the *tetragena* type, and in all cases there was a history of dysentery prior to the acute attack preceding admission. When no history of dysentery, but only of vague intestinal disturbance was obtained, the entamoebae were of the *minuta* type, and, as noted, remarkably constant in their morphology. In one case of *minuta* type infection there was no history of dysentery at any time.

CORRELATION OF THE TYPES OF ENTAMOEBAE

Whether a plurality or a unity of the species of the pathogenic *Entamoebae* of man is accepted, in studying many cases of amoebic dysentery, certain facts are found common to all infections with entamoebae whose cycles end in the production of four-nucleated cysts.

In the total observations on Types A, B, and C, it was found that at one time or another entamoebae of such type identical in morphology were met with in the same infection. These were the forms encountered in the stage intermediate between the large vegetative forms and the pre-cystic generation, and are represented in figs. 5-9.

I have no doubt, as noted, but that further investigation will show a Type A infection followed out to encystment. Types B and C, while differing in the development of the cycle, form cysts which morphologically cannot be distinguished, and also show similar morphology in the intermediate stage above mentioned. In mild and latent dysenteries the cysts are identical with those found at the close of a cycle of a Type B or a Type C infection, although the preceding cyst-forming generation may have been either of the *tetragena* or of the *minuta* type.

That environment, rather than plurality of species, appears to account for the diversity of morphology can also be demonstrated. The large vegetative entamoebae of Types A, B, and C, are never found except in pus or mucus. The stool itself may be formed, and not infrequently is, when one of these types is present, but if so, the entamoebae will be found only in bloody mucus on the exterior of the faecal material. The large vegetative entamoebae, while rarely associated with cysts, as in the beginning of relapse, are not found in the pre-cystic and cyst-forming generations, unless brought down from some intestinal ulcer, and then they will be associated with pus and bloody mucus.

Of the two types of pathogenic entamoebae found in mild or in latent dysentery, the morphology is often determined by the presence or absence of recent dysentery. When there is a history of recent dysentery, the entamoebae have for the most part the nuclei and cytoplasmic appearance of the small *tetragena* forms; when there is no history of recent acute dysentery, they show the characteristics of the *minuta* type.

If a plurality of species, based on morphology, is assumed, there must be in the Canal Zone, and elsewhere, a very high percentage of mixed infections, since in so many cases of amoebic infection there are present entamoebae of different types at one time or another. And if these different species exist, it is indeed singular that the end stage, the four-nucleated cyst, varies so little in each species as to appear morphologically the same, whether found after a Type B, a Type C, or a *minuta* type infection. All who in the past two years have followed the cycle of a pathogenic entamoeba to its conclusion, agree that the end stage is the same.

I have seen specimens of four-nucleated cysts from widely-divergent sources, and from pathogenic infections with histories of markedly different clinical courses, but in every instance the morphology of the cyst itself was the same.

On the other hand, if a unity of species is assumed, the variations in morphology and in the development of the cycles can be explained on biological grounds. The work of experienced protozoologists shows that the protozoa vary in morphology and in development not only in proportion to changes in the media, but also in proportion to the passing of the cycles through the various phases between youth and old age, as was shown in Part II, sections 1 and 3. The contents of the intestine are not the same in the acute stages of dysentery as in the convalescent stages, nor is it necessary to suppose that each cycle follows in development exactly the cycle of its predecessor. The same is true in malarial infections, in which variations from type in the same species are sometimes quite as marked as in the entamoebae, and have given rise to as many speculations as have the varieties of the latter organisms.

It is generally admitted that the primary factor in the causation of amoebic dysentery is the ingestion of the four-nucleated cysts. It is not believed, to the best of my knowledge, that pathogenicity at once follows the establishment of the infection. If the histories of my cases are correct only in general, and are not precisely accurate in individual instances, there has been none the less in the past two years in the Canal Zone a certain amount of entamoebic infection that had not taken on active pathogenicity, as manifested by symptoms of dysentery, prior to the finding of the entamoebae in the stools. In default of exact and controlled feeding experiments in man it is not possible to say how long an infection may remain latent, nor what are the conditions that bring about pathogenicity.

There are, as has been noted, many degrees of severity in amoebic dysentery. Severe cases are accompanied by great activity on the part of the entamoebae, as shown by the large numbers in which these are found, the persistence of the disease, and the extensive ulceration found at autopsy. The activity of the entamoebae in acute dysentery is further shown by the large size, the phagocytosis of erythrocytes, the penetration of the wall of the bowel, and above all by the many forms with two nuclei and nuclei in division.

In this stage of the cycle, whether the activity be primary or due to rejuvenation, as in relapse, the entamoebae belong to that phase of the cycle described by Calkins (1909) as the period of youth. 'In youth the organisms are at the period of greatest activity and pathogenicity, and there are fewer variations from type.' It is because of this activity that infections in this stage do not yield readily to rest in bed and a restricted diet, and the entamoebae persist with a definite type of nucleus, that of the *histolytica* variety.

It should be carefully noted that such activity may be due to greatly renewed vitality in an infection of long standing, and is not of necessity characteristic of a primary attack.

In older infections, although the symptoms at first may be quite severe, the strain is more mature, and the symptoms produced yield readily to rest in bed and a restricted diet. Also, a primary attack may be brought about by a renewal of vitality in a strain which has remained latent for a certain period, but for some reason cannot assume the pathogenicity characteristic of the very acute attack. 'When the organisms reach maturity there are changes in the cytoplasm, plastic in kind; the nuclei are more definite and typical; and conjugation takes place. Calkins (1909).' As noted, in all of my infections with the *tetragena* type, prior to encystment such changes as described by Calkins were clearly evident, except that conjugation was not observed. I do not know whether the renewal of vitality manifested by pathogenic activity, which is so characteristic a feature of amoebic dysentery, is due to conjugation or to some power inherent in the organisms. However this may be, at the stage of maturity, which is manifested by the appearance of entamoebae of the *tetragena* type, the nucleus is more definite than in the *histolytica* type, and the well-defined cyclical changes denote a maturer organization. I consider it probable also that whatever process it is that brings about the relative immunity against this type of infection, as shown by the ease with which it is controlled without medication, has to do with the development of the maturer type.

This is the stage also when the formation of the resistant forms, for the purpose of continuing the infection outside of the body, is to take place, since, as noted, and contrary to the experience of some others, in all of my *tetragena* type infections the exacerbation resulting in acute symptoms was shortly followed by encystment.

'Old age,' Calkins states, 'is shown by marked degenerative changes in the cytoplasm, such as vacuolization and degeneration, and the nuclei also degenerate. Unless there is in some way a rejuvenation of vitality, the cycle will end here, to be taken up later, under suitable conditions, by the resistant forms which have been developed earlier for this purpose.'

In the life cycle of the entamoebae the degenerating forms are most frequently encountered in the pre-cystic and encysting stages subsequent to an acute attack of dysentery. In these stages they are encountered in great numbers, and it is evident that only a part of the organisms go on to encystment. Yet not all of the vegetative entamoebae perish, as is evident from a consideration of relapsing cases. However, considered as an expression of the vitality of the cycle, the stage of encystment marks a temporary cessation of pathogenic activity.

I could not make sufficient observations in the intervals between attacks to state in what form the entamoebae continue the infection after the close of a cycle, one part of which was accompanied by pathogenic activity. Autopsy observations by Dr. H. C. Clark show that ulceration may exist at this time, unaccompanied by symptoms of dysentery, and, as noted, vegetative entamoebae of the *tetragena* type were found in the ulcers, when no cysts nor pre-cystic organisms were found in the stools. On the other hand, the *minuta* type of organism, both vegetative and encysted, is met with in cases with neither history nor symptoms of acute dysentery.

The above data show that if unity of the pathogenic species be accepted, variations from type must be recognized and accounted for. To accept a plurality of the pathogenic species one must admit that each species produces a separate clinical picture. The latter does not seem to me to be probable, since I find too many transition phases between the types described, occurring in similar clinical pictures.

However, as stated in Part I, I make no claim whatever to an expert knowledge of protozoology. The classification of species I leave to those better qualified to make it. My own belief is that there is but one species, which manifests itself according to conditions of vitality and environment in the forms which I have described.

THE SO-CALLED CHROMIDIA FORMATION IN THE ENTAMOEBAE

In many of the Protozoa the chromatin is not always confined to the nucleus, but passes out into the cytoplasm in the form of more or less minute granules which collectively are known as *chromidia*. For the small individual particles that constitute the chromidial mass Minchin (1912) has proposed the name of *chromidiosomes*.

'It is even found that in some species a true nucleus may be absent temporarily during some phases of the life cycle, all the chromatin being then in the form of chromidia, from which the nuclei arise by a process of condensation and organization of the chromatin in combination with the achromatinic elements. Such a condition may be regarded as a temporary reversion to a more archaic and ancestral condition, since . . . the Protista of the lower or bacterial grades of organization do not possess, speaking generally, a true nucleus, but only scattered grains of chromatin' (Minchin, 1912, p. 116).

Calkins (1909) states that the first definite observations upon chromidia formation were made by Richard Hertwig (1899). The function of this extranuclear chromatin was not made out until 1903, 'when Schaudinn worked out the origin and fate of similar masses of granules in several different kinds of sarcodina, and found that the nuclei of the conjugating gametes were developed solely from the extranuclear chromatin' (Ibid., p. 116).

In the meantime, however, Hertwig showed 'That in starving forms, and in forms that had been overfed, the nuclei all disintegrate and the chromatin contents become distributed throughout the cell body. The distributed chromatin thus formed was named by Hertwig, in 1902, *chromidien*, from which the term *chromidia* is derived, a term now universally employed by protozoologists. According to Hertwig, this latter material in *Actinospherium* cytoplasm is prophetic of the death of the animal' (Ibid., p. 116).

Calkins then states: 'Chromidia, in Hertwig's sense, is functionless extranuclear chromatin, but Schaudinn and others have used the term to designate sexual chromatin, which is equivalent to the chromidialnetz in Hertwig's terminology.' Calkins uses the term *chromidia* for the functionless extranuclear chromatin, and *idiochromidia* for that which has sexual function.

Concerning the chromidia found in the entamoebae, Whitmore (1913) quotes Hartmann (1912) as writing: 'They are true chromidia in the sense used by Richard Hertwig (i.e., functionless extranuclear chromatin. W. M. J.), as is shown by their staining and microchemical reactions.' They 'have their origin in the nucleus,' but 'increase enormously in size and number in the cytoplasm.' Whitmore then states: 'As long as that opinion is held, a difference in the staining between the chromidia and the chromatin in the nucleus will speak rather for some fallacy in the staining method used, unless carefully controlled by a number of staining reactions.'

As to the origin, purpose, and fate of the chromidia, whether sexual or functionless, in Protozoa other than the *Entamoebae*, I know very little. I have, however, made a prolonged study of what are called chromidia in the *Entamoebae*, and my conclusion is that they are derived from the cytoplasm by a process of condensation in the latter, and have nothing whatever to do with nuclear chromatin. Although by analogy the appearance in the *Entamoebae* of a material similar in staining and in microchemical reactions to what are interpreted as chromidial masses in other Protozoa may justify the belief that true chromidial masses are to be found in the *Entamoebae* also, I desire to call attention to the grave errors resulting from the application, in the study of the *Entamoebae*, of interpretation by analogy instead of by fact. It was analogy that led Schaudinn into error in his interpretation of the phases of encystment in *E. coli*, and in his exposition of the cycle of *E. histolytica*. Analogy, as Prof. Amico Bignami observed in his criticism of parthenogenesis in the malarial parasites, is not of value unless supported by correlative data, although it takes on importance when such data are available. But the data must justify the analogy, not the analogy the data.

It is because the data collected by me do not justify the belief that what are called chromidia in the *Entamoebae* are of nuclear origin, that I cannot accept the prevailing belief with respect to them.

Hartmann (1912) states: 'After the different nuclear stains applied (carmine, hematoxylin, safranin, methylene blue, methyl green) they (the chromidia) take the same colour as does the chromatin of the nucleus. They show neither the staining nor the reactions of metachromatinic bodies (volutin), nor those of glycogen, which of late have been demonstrated for other so-called chromidia' (p. 177).

Now, all of the stains used by Hartmann and others to demonstrate an identity of chemical structure between nuclear chromatin and chromidia are monochrome stains, and as such dye in various hues of the same colour all of the component parts of the entamoebae. Iron hematoxylin, for instance, as commonly used, stains the entire organism at first a uniformly deep colour, and it is because the chromatin is more retentive of the stain that after differentiation it is demonstrated in a darker shade. But substances other than chromatin, and in no way related to it, are equally retentive of the stain, such as the erythrocytes, and in some instances the cytoplasm itself, as is shown by the difficulty of obtaining good differentiation in some phases of the pathogenic entamoebae, and especially in the pre-cystic and cyst-forming generations of *E. coli.*

The same fault is to be found after the use of carmine, safranin, methylene blue, and methyl green. None of these stains have a specific value in staining chromatin. They may demonstrate plainly enough the chromatin itself, but they stain also, in varying hues of the same colour as the chromatin, the achromatinic structures of the nucleus, the cytoplasm, and foreign material. Some of the finest examples of the achromatinic network of the nucleus that I obtained were after the use of methyl green, which some authorities indicate 'as a most distinctively nuclear stain.'

An excellent account of the many fallacies that result from the identification of chromatin by monochrome stains is given by Minchin (1912), pp. 68-70.

From these data it is evident enough that similarity in staining reactions after the monochrome stains is no proof of identity between the chromatin and the chromidia.

According to Minchin (1912): 'The chromatin may itself give rise to other substances of an achromatinic nature, and probably of simpler constitution, by a process of breaking down of its complex substance; and also there may be present in the cell various substances very similar to chromatin in their properties, representing stages in the building-up of the complex material of the chromatin-substance. In one or the other of these two ways it is possible to account for bodies in the cell known by various names, such as "metachromatinic grains," "chromatoid grains," and so forth—bodies which are often mistaken for true chromatin, but which must be carefully distinguished from it, just as metaplastic bodies are to be distinguished from protoplasm. Among such bodies must be mentioned especially the so-called "volutin-grains," which have attracted much attention. . . . The volutin grains resemble chromatin in showing affinities for so-called nuclear stains, which they hold more firmly than the chromatin itself, when treated with reagents that extract the stain' (pp. 67, 68). Minchin further states that the volutin grains have a totally different nature from the chromidia, with which they are often confused on account of their similarity in appearance and in staining reactions.

Thus is demonstrated a conflict of authority as to the staining reactions of the metachromatinic bodies, Hartmann holding that these do not react as does chromatin after the 'nuclear stains,' and Minchin, that in some instances they stain more intensely than the chromatin itself. It appears to me that the evidence which I shall now present will demonstrate that the so-called chromidia are of cytoplasmic origin, since there are stains that will show a difference in reaction between the chromatin of the nucleus and the cytoplasm.

As noted, the monochrome so-called nuclear stains do not afford definite information as to what is chromatin and what is not. It is different when polychrome stains that have a definite reaction to the chromatin of the nucleus and to the cytoplasm are employed. It is of interest here to note that especially after wet fixation, there is very little mention made of the employment of polychrome stains in the study of the *Entamoebae*.

After staining with Hastings and Gimesa, in dry-fixed preparations the chromidia take a dark blue stain. It was formerly believed that the red or purple masses found after this method in the cytoplasm of the entamoebae represented chromidia of nuclear origin, but later research has shown that this opinion was erroneous. The reaction and the structure of the chromidia after this staining method indicate that they are of cytoplasmic origin. In the cysts the chromidia show as blocks and spindles of finely granular structure staining an intense dark blue, while the erythrochromatin of the nuclei appears as a dark red or purple, in the same shape and form in the resting nuclei as seen after wet fixation.

After wet fixation and Mann's stain the nuclear chromatin and the chromidia react as structures of different chemical composition. The chromatin stains in shades of red and orange, the chromidia, in shades of lilac and blue. These reactions are constant, and are identical after all of the methods of fixation employed by me. It appears from this that a polychrome stain, which separates specifically the chromatin from the chromidia, is of more value as evidence of dissimilarity than a monochrome stain, which gives only hues of the same colour, is of value as evidence of identity.

In view of this the inference is justified, at least for the present, that the fallacy referred to by Whitmore lies in the monochrome rather than in the polychrome staining method.

Neither does the morphological evidence of the production of the chromidia from the nuclear chromatin appear to be sufficiently substantiated. Pictures are given of nuclei surrounded by rods and granules of chromidia, or of nuclei which apparently are extruding chromatin into the cytoplasm, and this is claimed as the beginning of chromidia formation, and as proof of its nuclear origin. It may be granted that some nuclei disintegrate and extrude their contents into the cytoplasm, but the polychrome stains show that this chromatin either changes its chemical composition almost at once, which is not admitted by the advocates of nuclear origin of the chromidia, or else that such extruded chromidia form but a very small proportion of the total amount, which I believe to be correct. The position of the rods and granules around the nucleus is no evidence at all that they are of nuclear origin, since it can be demonstrated that in very many entamoebae the nucleus is at one pole and the 'chromidia' are at the other. It does not appear to me, from a study of many examples of the so-called beginning chromidia formation, that the relative positions within the entamoebae of the nuclei and the first formed granules of chromidia are constant. Further, even in the earliest stages of formation, the chromidia react constantly and definitely, and in a manner different from that of the nuclear chromatin, to the polychrome stains.

Nor does the nucleus constantly lose its chromatin in proportion to the amount of chromidia formed, as some assert. Hartmann and Whitmore (1912) state that no matter how heavy the chromidia formation in the early stages of encystment in *E. coli*, the nucleus is always fully individualized. It is true that in many vegetative entamoebae full of chromidia the nucleus is poor in chromatin, but it is equally as poor, in the same preparation, in many entamoebae in which there are no chromidia. Also many entamoebae rich in chromidia show nuclei rich in chromatin as well. I do not know why these facts have hitherto been overlooked, but I am certain that there is no constant relation between the amount of chromidia in the entamoebae and the amount of chromatin in their nuclei, after either monochrome or polychrome stains.

Finally, with respect to the premise that the chromidia increase by multiplication of the chromidiosomes, what happens in moist chamber preparations should be considered. In these after an exposure from fifteen minutes to two hours, many of the entamoebae are found to be filled with chromidia. The relation between such chromidia formation and that observed during pre-encystment is not clear, but one would not suppose that a dying organism, such as is found in a moist chamber preparation, could produce so great an amount of chromatin from its nucleus. Structural alterations in the cytoplasm, however, would account for the formation of the chromidia in such cases.

Whitmore (1913) states: 'When a protozoal cell is dying, chromatin is thrown out from the nucleus in the form of chromidia, which are to be thrown off from the cell.' But the chromidia found under such conditions do not react after polychrome stains as does the nuclear chromatin, either at the beginning of chromidia formation, or when the cell is stuffed with chromidial masses, and this discrepancy will not be accounted for by assuming a fallacy in the staining method.

My observations lead me to believe that the so-called chromidia are found under two conditions: —

1. When certain of the pathogenic vegetative entamoebae degenerate, as in moist chamber preparations. Chromidia are by no means found in all of the entamoebae of moist chamber preparations.

2. In the pre-cystic generation of the pathogenic species, certain of the entamoebae, apparently most of those that are going on to cyst formation, show chromidia formation in varying degrees. Not all of the entamoebae that pass into cyst formation have chromidia, since not a few pre-cystic organisms and many uninuclear cysts show no chromidia formation. Chromidia formation, in my experience, is not associated with the degenerative changes in the nuclei and the cytoplasm seen in so many entamoebae of the pre-cystic generation. In *E. coli* chromidia formation is very seldom, if ever, found before encystment.

Undoubtedly there is much that is yet obscure in this process. I am not aware of any one hypothesis as to the origin, function, and fate of the chromidia in the *Entamoebae* that can at present be fully substantiated. Morphologically, a series of entamoebae showing all the phases of cyst formation without chromidia formation can readily be demonstrated, but whether this would be a true biological series, or only an arbitrary one representing phases in which there is no chromidia formation, I cannot at present state. It is true, as far as the pathogenic species is concerned, that chromidia formation in the pre-cystic and encysting generations is the rule, while in *E. coli* the process takes place irregularly.

But whatever may be the true function of chromidia formation in the *Entamoebae*, I do not believe that the hypothesis of nuclear origin can be sustained in view of the data that I have brought forward.

PART IV

THE TECHNIQUE OF VARIOUS METHODS USED IN FIXING AND STAINING THE ENTAMOEBAE

Dry fixation and Staining. A smear is made by placing a small portion of the material towards the end of a slide, covering this with another slide and spreading it, and then drawing the two slides apart lengthwise. They are next dried, and may be fixed in absolute methyl alcohol.

The smear is then stained by one of the modifications of the Romanowsky method. I have obtained my best results after the following method, which was originated by Dr. S. T. Darling some years ago for the study of autopsy preparations in malaria.

The air dried slide is fixed for about five minutes with undiluted Hastings' stain, a modification of the Romanowsky used in Ancon Hospital. Distilled water is then added until no more will remain on the slide. The preparation is stained for at least an hour, preferably two to three, or even longer, more water being added from time to time to prevent drying. It is also of value to add occasionally a few drops of stain. When staining is completed, rinse the specimen in tap water, and dry.

The slide is now placed in any good Giemsa solution, and stained overnight. It is rinsed next in tap water, and dried, after which it is differentiated by dipping it a few times in 60% alcohol containing about 1 % aqueous solution of ammonia.

This method gives excellent preparations after dry fixation. The double staining I have repeatedly found to be more satisfactory than either of the stains used alone, especially to demonstrate the erythrochromatin in the nucleus. I am aware that dry fixation destroys certain details shown by wet-fixation methods, and distorts the nuclear structure. None the less, after a careful comparison for the last year and a half of various methods of fixation and staining, I am convinced that dry fixation followed by staining as indicated gives results as constant in appearance as does any wet-fixation method. The nuclear contents may be distorted, as noted, but the distortion is uniform and characteristic, and the cytoplasm of the entamoebae stains quite as satisfactorily as after wet fixation. The method certainly is not of any value in studying the reproductive phases in the cycle, but I claim for it that it is reliable in so far as the identification of *Entamoeba coli* is concerned, as shown in the illustrations of entamoebae stained by it.

WET FIXATION AND STAINING METHODS

The purpose of what is commonly called 'wet fixation' is to preserve as nearly as possible unaltered the structure of the nucleus and the cytoplasm of a cell. Drying distorts and breaks up the nuclear chromatin, and destroys the alveolar structure of the cytoplasm. For this reason, *from the moment that the smears are made until they are finally mounted they must be kept moist, and not allowed to dry, even slightly.* The changes from one fluid to another must be made quickly, without much draining of the preparations, even at the risk of carrying traces of one mixture into another. This latter frequently necessitates the use of two portions of certain of the fluids, the first to wash out all traces of the preceding fluid, and the second for the proper purpose.

It is very convenient to have at hand an ample supply of staining jars, so that a clean set of these may be used with each series of fluids, instead of having to clean the jars at every change. Such jars are inexpensive, and may be obtained from any dealer in microscopical supplies. They should hold at least 10 c.c. of fluid for each slide put in them, and be provided with well-fitting covers.

There is some difference of opinion as to whether coverglasses or slides should be used in the preparation of specimens. Each method has its advantages, but personally I prefer to use slides, as they are far easier to handle, and I do not know of any very satisfactory staining jar for the use of coverglasses. There are occasions, however, when coverglasses must be used, as will be explained. Also, for the transportation of specimens in alcohol, before staining, coverglasses are more convenient, as they may be placed one against another in a bottle containing the fluid.

1. *Preparation of specimens.* The material is rapidly spread,

as thinly as possible, on a coverglass or slide, and dropped immediately into the fixing fluid. One will often have much difficulty in doing this, for a variety of reasons. If the material used is from the mucus of a stool, frequently it will not spread easily, and thin smears are essential to good work. If slides are used, a camel-hair brush may be tried, which will often make a very good smear. If this is not successful, move the material on the slide about with a platinum loop, and flatten out each bit as rapidly as possible. Experience is the only guide in making such preparations. If coverglasses are used, place the material on one, cover it with another, and after the material has spread, draw the coverglasses apart as in making a blood film, and drop them face (material) downward on the fixing fluid, where they will float. Sometimes good results can be obtained by placing the material on a slide, covering it with a coverglass, and sliding the latter off. If square coverglasses are to be used for mounting, draw the coverglass at right angles to the slide. If rectangular coverglasses are to be used, and these are preferable, draw the coverglass lengthwise. In either case, most of the material will remain on the slide, and some of it will be spread out in a thin film. When the smears are to be made on coverglasses the latter must be thin, but if the smear is made on a slide with the aid of a coverglass, a thick coverglass should be used to spread the material, as thin ones very often break.

Sometimes all of these methods will fail in producing good preparations; then the best one can do is to use a platinum loop, and tease out the material as thinly as possible, later removing the thick masses that remain after fixation.

If the stool is hard, a portion should be placed in a watch crystal, and mixed with enough normal salt solution to make a paste. The mixture should then be spread as will be described. If the stool is semi-fluid, there will be no difficulty in making good preparations. The best method to my knowledge is that recommended by Elmassian (1909). With a soft camel-hair brush a little of the material is spread as rapidly as possible, and as thinly, on the coverglass or slide. When the entamoebae are not plentiful in the material, dilute the latter with warm normal salt solution and centrifuge. Pour off the supernatant fluid, and make preparations from the sediment. Another method, which gives very thin and even smears, is to spread such material (semi-solid) between two coverglasses, or on a slide with the aid of a coverglass. When the entamoebae are quite plentiful this method can be very highly recommended, but if the organisms are scarce, it is better to use the camel-hair brush, and to make thicker preparations.

When the stool is liquid, very often a smear that will adhere cannot be made unless the stool is centrifuged, and the sediment used. Mucus threads, which often contain very many entamoebae, are sometimes found in the sediment. These threads can usually be spread out with a platinum loop or a camel-hair brush.

Quite frequently the entamoebae may be found only with difficulty. This is particularly true in liquid stools. Such stools should be carefully searched for small particles of mucus, particularly bloody mucus, and these should be picked out with a flat pointed forceps.

In making a specimen, it is necessary to remember that it will have to be examined at times in the staining process, and, as noted, the preparation must always be kept wet. For this reason the size of the smear should not exceed that of the coverglass to be used for mounting. Rectangular coverglasses permit of the use of a larger amount of material, and so are preferable, especially for mucus preparations.

2. *Fixation.* As soon as the smear is made, the preparation must be placed at once in the fixing fluid. The thin smears, especially those made on coverglasses, dry very rapidly, and quick work•here is absolutely necessary. Slides are placed in the fixing fluid with the edges or ends up; coverglasses are floated with the smear side downward for a minute, and then turned over and sunk. It frequently happens that when the preparation is placed in the fixing fluid, the material floats off the slide. One must then change to coverglasses, on which material will often remain when it will not stay on a slide. If the material will not adhere to the coverglass, all that can be done is to wait for other stools, or to try to change the consistency of the stool by altering the diet, by adding soft food, such as eggs, rice, and bread. As a rule, by use of one of the methods in the preceding section the material can be made to adhere, but glairy mucus always gives trouble, and often one

must be content if one succeeds in getting two or three specimens from a dozen or more attempts.

Many fixing methods have been advocated by different authors, and some particular advantage is urged for each. The appearance of the entamoebae, or of any other cells, varies with the methods used, and from this it is perhaps logical to infer that the truest interpretation will be attained by a composite picture. Such a method, however, is suitable only for research work, and not for routine. For the latter, it is the consensus of opinion that one method only should be used for a differentiation of species. An excellent account is given by Dr. Gustave Mann of the various fixing fluids and their reactions to tissue cells, in his book, *Physiological Histology*. The technique of fixation for the *Entamoebae* differs somewhat from that employed for tissue cells, and the various methods should be modified accordingly. I have tried many fixing methods, and have chosen the following for my own use, as giving the most constant results. Unless otherwise specified, the word 'alcohol' refers to ethyl alcohol.

(*a*) Schaudinn's Fluid. This excellent fixative is the standard in the study of the *Entamoebae*. Most of the figures given by authoritative writers are drawn from specimens fixed by it. Originally it was composed of one part of absolute alcohol to two parts of saturated aqueous solution of sublimate (bichloride of mercury). It is used mostly at present as one part of absolute alcohol to two parts of a normal saline solution saturated with sublimate, plus from 2½% to 5% of glacial acetic acid. The saline solution keeps better than does the aqueous, while the acetic acid improves nuclear fixation. Absolute alcohol, however, cannot always be procured when wanted, and is very expensive. I frequently substitute for it the ordinary commercial alcohol, which is about 96% pure, using slightly more (2 to 3 c.c.) of the alcohol and slightly less of the sublimate solution than given in the first formula. I do not find that this impairs in any way the fixing quality of the mixture. The alcohol and the sublimate solution should be mixed shortly before using, and the acetic acid added immediately before, especially when warm solutions are used. Some prefer to use two portions of the fixative. The first is heated to about 60° C., and the second to about 37° C. The preparation is plunged into the hot fluid, and immediately withdrawn and placed in the warm fluid, where it remains five minutes. Fixation in the fluid at 37° C. alone for five minutes, or in a fluid at room temperature from five to ten minutes, not longer, gives excellent results, and is often more convenient, since the hot fluid frequently removes material from the slide. The time of fixation is most important, for if it exceeds the limits given, distortion will almost always follow.

After fixation the preparation is placed at once in 50 % alcohol, where it remains for ten minutes. It is then transferred into 70 % alcohol, for at least fifteen minutes. If it is not desired to go on to staining at once, place the preparation in 80 % alcohol, which should be changed at the end of twelve hours, and preserve in this until ready to stain. The sublimate is discharged in this alcohol, as well as in the lower alcohols, and the preserving fluid should be kept as free from it as possible, otherwise crystals of sublimate will form on the specimen.

If it is desired to proceed at once to staining, the sublimate must be discharged with iodine alcohol. This is made by adding to 70 % alcohol about 1 % of Lugol's solution, and enough iodine to give a brown colour, or the Lugol's solution may be omitted. The preparation is placed in this iodine alcohol for half an hour, placed in fresh 70 % alcohol until the material is no longer yellow (about ten minutes) and hardened in 80 % alcohol for at least fifteen minutes. It is then transferred to 96 % alcohol for ten minutes, and to absolute alcohol for ten minutes more. It is then taken down through 80 % alcohol five minutes, 50 % alcohol five minutes, to distilled water, and further treated as described in the section on staining. The alcohols should be kept in separate bottles, and can be used repeatedly.

Some authorities prefer to omit the hardening in the higher alcohols, using only the 80 % for about half an hour, and then go through 50 % alcohol for five minutes to distilled water, but the use of the higher alcohols gives a clearer definition to cytoplasm and nuclear structure after staining. Slides preserved in 80 % alcohol for several days are usually sufficiently hardened, although some recommend the hardening in 96 % and absolute alcohols always prior to staining. Personally, I believe that this is slightly better.

(*b*) Zenker's Fluid. This well-known fluid is also an excellent fixative for the *Entamoebae*. It is made as follows:—

Bichromate of potassium	2·5 grammes.
Sulphate of sodium	1 gramme.
Bichloride of mercury	5 grammes.
Glacial acetic acid	5 c.c.
Distilled water ....ad.	100 c.c.

'Dissolve the bichloride of mercury and the bichromate of potassium in the water with the aid of heat, and add the sulphate of sodium. It is not advisable to add the acetic acid to the stock solution, but only the proper proportion just before use. Use 5 c.c. of the acetic acid to 95 c.c. of stock solution' (Mallory and Wright).

Specimens are fixed in the solution at room temperature for five minutes, transferred to 50% alcohol, and treated further as described above after Schaudinn's fluid. It is not necessary to wash the slides in water after fixing, although Walker (1911) advises this, using distilled water, before dehydrating and hardening in the alcohols.

Zenker's fluid shows very well the segments of chromatin under the nuclear membrane, especially when used before Mann's stain. Before finally placing in the preserving alcohol, one should take care that the fixative no longer colours the preceding alcohol.

(*c*) Bouin-Duboscq's Fluid. This fixative appears to be a favourite with French workers, as many of their drawings refer to its use. It is recommended by Elmassian (1909, also in a personal communication) as especially valuable when used as a preliminary to Mann's stain. Dr. Elmassian sent me the following formula for its composition:—

Picric acid crystals	1 gramme.
Glacial acetic acid	10 c.c.
Formol (commercial formalin)	50 c.c.
75% alcohol	150 c.c.

Chatton and Lalung-Bonnaire (1912) use a slightly different mixture:—

Picric acid crystals	1 gramme.
Glacial acetic acid	15 c.c.
Formol	60 c.c.
80% alcohol	150 c.c.

As with Zenker's fluid, it is better to make a stock solution without the glacial acetic acid, and to add this just before using.

Fix from five to ten minutes, not longer, and transfer directly to 70 % alcohol. This is changed two or three times, until no more picric acid is discharged into the alcohol. The first change should be made in about ten minutes, the second half an hour later, and the third about twelve hours after the second. Dr. Mann advises (personal communication) the immediate transfer, after fixing, into absolute alcohol, which is changed several times until the picric acid is discharged.

(*d*) Flemming's Fluid. I have not used this myself, but have seen excellent results from its use. Dr. S. T. Darling used it to fix smears from his artificially infected cats, and the karyosomes were very beautifully demonstrated. The formula is:—

Osmic acid, 2 % aqueous solution	4 c.c.
Chromic acid, 1 % aqueous solution	15 c.c.
Glacial acetic acid	1 c.c.

Specimens are fixed from five to fifteen minutes, and washed in running water for half an hour. If the latter is not available, then several changes of water must be used. Mallory and Wright (1908) advise that the reagents be kept separately, and mixed just before use. After washing thoroughly in water, harden in alcohols of increasing strength, as after Schaudinn's fluid.

(*e*) Mann's Fluid. Dr. Mann (personal communication) is of the opinion that his osmic acid-sublimate mixture will give good nuclear and cytoplasmic pictures. I have not as yet had opportunity to try this, but give the formula here, as Dr. Mann is a well-known authority on all that pertains to fixing and staining solutions.

Saturated sublimate in $\frac{3}{4}$ % sodium chloride solution	50 c.c.
1 % osmic acid aqueous solution	50 c.c.

Fix in the dark for fifteen minutes. Transfer to 50 % alcohol, in the dark, change this twice in the first day, and then take through 60 %, 70 %, 80 %, 90 %, and absolute alcohol, and back to 80 %. However, if the reagents are at hand, Dr. Mann (personal communication) advises for the removal of the osmium after any fixing fluid in which it is employed the following:—

Treat sections with $\frac{1}{4}$ % solution of potassium permanganate for five minutes. Decolorize the brown sections in a saturated watery solution of sulphurous acid, 1 part, and normal saline (0·75 NaCl), 9 parts. Transfer them to ascending alcohols, as given after Schaudinn's fluid.

(*f*) Dr. A. Gauducheau has sent me the following as the fixing fluid used by him in the preparation of the specimens from which his illustrations accompanying this paper were drawn. As can be seen, it is a combination of the fluids of Mann and Flemming.

Chromic acid	1 gramme.
Glacial acetic acid	1 c.c.
2 % aqueous solution of osmic acid	25 c.c.
Saturated aqueous solution of sublimate	175 c.c.

Fix for thirty minutes, and treat after one of the methods given above for hardening after osmic acid mixtures.

STAINING METHODS

Fixation is largely a mechanical process, and if one keeps in mind the cardinal principles of using fresh material, making thin smears, keeping the specimens from drying at any stage, and removing metallic salts such as the sublimate by the methods indicated, one should obtain satisfactory results in this part of the technique. Staining, however, is largely a matter of personal judgment, and many of the variations and peculiarities which in the past have been used as criteria for the determination of species are, I am convinced, no more than results of individual differences in staining. It appears to me that no standardization of staining methods can be accurately otained, since in some respects, as will be seen, each specimen must be handled separately. In practically all methods of staining, it is necessary first to overstain, and then to decolorize, or differentiate. Proper decolorization depends upon experience, and can be obtained only after considerable practice. Many staining fluids are in use, and I shall give only those which are standards, or which personally I have found to give good results. Each method has advantages, and, as far as I know, there is none that will demonstrate all the features which should be studied. However, one stain should be selected as a standard, and the others used to show variations from this. Any of the staining methods described below may be used after any of the fixatives already given.

For each of the staining methods the following process is used as a preliminary. The preparations should be freed from alcohol, which is done by taking them from the preserving alcohol to 50% alcohol for five minutes, and then washing them carefully in distilled water for five minutes. It is of advantage to use two changes of distilled water for five minutes each.

(*a*) *Iron hematoxylin Stains* (*Heidenhain's Method*). These stains are used essentially to demonstrate nuclear structures, but properly applied, they will also give very good pictures of the cytoplasm. The basic principle is to mordant with a solution of some iron salt, which combines more strongly with nuclear than with cytoplasmic structure. The excess of mordant is then removed with distilled water, and the specimens stained in the hematoxylin solution. This latter combines with the iron impregnated cell contents, in proportion as they have combined with the mordant. As the nuclear contents take the mordant more strongly than does the cytoplasm, they are the more heavily stained. After staining, the preparation is decolorized in the same solution used for the mordant, and thoroughly washed in running tap water until all traces of the mordant have been removed, for if any of the latter remains in the preparation, decolorization will continue.

Heidenhain's Iron Hematoxylin Method. Fixation with Schaudinn's fluid and staining by this method is universally recognized as the standard in the preparation of specimens of entamoebae, to which all other methods are referred. There are several variations of this method, depending on the time of mordanting and staining, and the strength of the solutions used. The method given here was sent to me by Major Eugene R. Whitmore, Medical Corps, U.S. Army, who learned it in Prof. Hartmann's laboratory in Berlin.

The specimens are taken from distilled water into a $3\frac{1}{2}$% solution of iron-alum (Ferric Sulphate of Iron and Alum), where they remain from four to eight hours, not longer, or the mordant will macerate the delicate cell structures.

The specimens are next rinsed by dipping twice in distilled water, preferably in separate vessels, and are carried into the hematoxylin staining solution, where they remain from twelve to forty-eight hours, preferably thirty-six to forty-eight. The staining solution is made as follows:—

Hematoxylin	1 gramme.
Absolute alcohol	10 c.c.
Distilled water	90 c.c.

The hematoxylin is dissolved in water by the aid of heat, and when the solution is cold, the absolute alcohol is added. The solution is then ripened for at least four weeks, in a dark glass bottle lightly stoppered with cotton. After ripening, it should be tightly corked. Before using, dilute one-half with distilled water, and the diluted solution is used for staining.

After staining, take the preparations one at a time through the next steps. Wash the preparation in two changes of distilled water, to remove the excess of hematoxylin, and place in the same solution that was used for the mordanting. In this iron-alum the preparation should be moved at frequent intervals. At first very black clouds will come off, which will dissolve in the iron-alum, but in a short while, from thirty seconds to two or three minutes, only faint clouds will come off. When this stage is reached, wash the preparation first in distilled water, then in a large dish of tap water, which stops further decolorization, and mount in water, with a coverglass, for examination. Use a high dry power objective, and a high power eyepiece, a water immersion lens will be found extremely useful for this. If the preparations are on coverglasses, these can be be placed face downward on the slide. (Major Whitmore, who uses coverglasses, makes two little ridges of shellac on a slide, about the width of a coverglass apart, fills the space between the ridges with iron-alum, and watches the decolorization from the beginning.) The entamoebae will appear in various stages of decolorization, from a deep black to a light grey, and the nuclei will be stained darker than the cytoplasm. Several organisms should be examined, and if in most of them the nuclei stand out well defined, with definite chromatinic segments or granules and well-defined karyosomes or centrioles, decolorization is complete. The preparation is then washed in distilled water, washed again in the large jar of tap water, and placed in a staining jar with tap water in it, and another preparation is taken from the stain, and put through the same process. Unfortunately, it only seldom happens that this result is attained on the first examination. As a rule, the nuclei are over-stained, and further steps must be taken cautiously, as the end stage is quickly reached, and over-decolorization may result. If the nuclei are over-stained, rinse the preparation in distilled water, place in the iron-alum solution, putting a coverglass over the material. Quickly wipe the iron-alum solution from the back of the slide, and put under the microscope. The entamoebae are then observed until the nuclei become clear, when the preparation is rinsed in distilled water, again in tap water, and put in the staining jar with the others. Some prefer to watch the decolorization from the start, by mounting in the iron-alum solution. This works very well when one is using coverglass preparations, and a slide with two ridges of shellac, as Major Whitmore does; but if the material is on a slide, and the preparation is mounted at first in iron-alum, and covered, without a preliminary decolorization in the staining jar containing the mordant, the excess of stain will darken the fluid between the coverglass and slide, and one cannot follow the process. One must be careful not to decolorize in the jar of mordant until clouds are no longer given off, otherwise over-decolorization will surely follow. A little experience will soon enable one to tell when to examine the slide. When all of the preparations have been decolorized, place the jar containing them in running tap water for at least fifteen minutes, better for two or three hours.

The preparations are now ready for dehydration. Place in 50 % alcohol, ten minutes; 70 % alcohol, ten minutes; 80 % alcohol, ten minutes; 96 % alcohol, two changes, one five minutes, one ten minutes; absolute alcohol, at least two changes, one ten minutes, and the other the same, or for fifteen minutes. Clear in xylol, two to three changes, as often in the first change the xylol clouds. The preparations are then mounted in acid-free xylol balsam. Thin slides and coverslips must be used, especially if one works with apochromatic lenses.

There are several points of practical value in the use of this stain, which I have found in my experience. If, for any reason, there is a scarcity of the iron-alum solution, keep one portion for mordanting, and the other for decolorizing. In this way the solution may be used repeatedly. There is no other advantage in this, however, as the iron-alum salt is cheap, and where it can be easily obtained, it is better to use the solution once for mordanting, and once for decolorizing, and then to discard it.

The hematoxylin solution should not be thrown away after using. Filter the used solution into a second bottle, and next time use 50 % of this, 25 % of stock solution, and 25 % distilled water. Filter this into the second bottle, and continue until the original stock solution is exhausted. Then begin with new stock solution, using each time one-half of the old, and a 50 % solution of the stock mixture. The stain improves greatly with this continued use, and becomes more definite. I have on hand now four bottles of hematoxylin, the oldest having been used with each of the other three. If, for any reason, there is no fresh ripened stock solution, the filtered and used solution may be used without dilution, and filtered back into its bottle. When two bottles of old and newer solution have been thus made up, use one-half of each bottle until both bottles are exhausted, each time filtering into a third bottle, which must, of course, be large enough to hold the contents of the two, or else use separate bottles, then begin with the fresh stock mixture, as indicated. My oldest hematoxylin has been used fifty or more times, and is still excellent. I am indebted to Major Whitmore for this valuable information.

It is preferable to begin the use of this stain with a few preparations only. After experience has been attained in decolorizing, one can save time by taking the preparations one after the other from the jar of stain, rinsing them as directed, and placing them together in the iron-alum solution. The preparations should be lifted and moved constantly, and when each gives off only a faint cloud of stain, wash it in the large jar of tap water, and place in the staining jar with tap water. Then, one at a time, rinse the specimens in distilled water, place in the iron-alum solution, take out at once, mount with a coverglass, and watch the decolorization. It is my experience that unless the specimens are decolorized individually, and the process controlled by microscopic examination, the result will most often be a failure. I have had many failures in making preparations by this and other methods, and practically all have been due to attempting to judge the degree of staining macroscopically, when the preparations were either under- or over-decolorized.

Trouble in decolorizing, when it is necessary to extract all the colour from the cytoplasm in order to render the nucleus visible, is generally due to too long mordanting, and the time of this should be shortened. Sometimes the nuclei retain the colour, due to physiological or pathological changes in the organisms, and a distinction must be made between such an occurrence and over-decolorization of the nuclei due to faulty technique.

The slides should be placed end or edge up in the staining fluids, otherwise too heavy a precipitation will take place on the material.

If a specimen is over-decolorized, and the fault is detected before the next step, the slide should be replaced in the staining fluid for half an hour or so, and then decolorized again.

Heidenhain's method gives a more precise nuclear definition than any other, but it requires much practice to obtain preparations that show not only good nuclear definition, but the structure of the cytoplasm satisfactorily as well. The older the stain, especially when it has been used repeatedly, the better the picture. The principal objections are the necessity of a thoroughly ripened hematoxylin solution, and the length of time needed for mordanting and staining. This latter has been overcome by more recent modifications, to which I shall presently refer, but these do not, in my experience, give as delicate nuclear detail as does the original process.

Heidenhain's Iron-Hematoxylin Method may be summarized as follows:—

1. Distilled water to remove all traces of alcohol.
2. Mordant four to eight hours in a 3½ % aqueous solution of iron-alum (ferric sulphate of iron and alum).
3. Rinse quickly in two changes of distilled water.
4. Stain twelve to forty-eight hours, preferably thirty-six to forty-eight, in a mixture of equal parts of stock 1 % hematoxylin solution, made as directed above, and distilled water. Later use filtered and stock solution as follows:—50 % stock solution, 25 % filtered solution, and 25 % distilled water, or half filtered solution and half stock solution without dilution.
5. Rinse in two changes of distilled water.

6. Decolorize in the same solution as used for mordanting
7. Rinse in distilled water.
8. Wash in tap water, preferably running, for at least fifteen minutes to half an hour, or longer.
9. Dehydrate in ascending alcohols.
10. Clear in xylol.
11. Mount in acid-free balsam.

Mallory's Chloride of Iron-Hematoxylin Stain. This is a valuable stain for quick work, and is quite accurate for identification, although the definition is not as sharp as by the Heidenhain method. I use it constantly to determine the number of entamoebae, by staining one or two preparations out of the total, to see if it is worth while to stain the others, for not infrequently only a part of the material originally smeared on the slide remains at the time of staining. Staining jars are not needed, except for decolorization, and the preparations may otherwise be treated throughout on two glass rods over a dish, as in the staining of blood films by one of the usual methods. Ripened hematoxylin is not necessary, as the solution is made just prior to staining. One must be careful, however, in changing the solutions, that the preparation does not dry.

1. Distilled water.
2. Place the preparation on the glass rods, or over a jar, and pour on at once a 10% aqueous solution of ferric chloride, enough to flood the preparation. Mordant in this ten to fifteen minutes.
3. Drain the slide, and pour on at once a 1% aqueous solution of hematoxylin, prepared as directed below. This turns black immediately, as the hematoxylin is precipitated, and should be poured off, and fresh solution added until the slide is flooded. The preparation should then be visible through the fresh solution. Stain five to ten minutes.
4. Wash thoroughly in two changes of distilled water.
5. Decolorize in a $\frac{1}{4}$ to 1% solution of the ferric chloride, following the principles of decolorization given in the description of the Heidenhain method. The strength of the decolorizing solution should be varied according

to the time of mordanting and staining. It is best to use about a ½% solution to begin with, and to increase the strength of this if necessary. If the decolorizing solution is too strong, the entamoebae will lose their colour too quickly.

6. Rinse in distilled water.
7. Wash in tap water (preferably running) for at least fifteen minutes, dehydrate in ascending alcohols, clear in xylol, and mount in acid-free balsam.

The hematoxylin solution is conveniently made by heating a test tube full of distilled water, and dissolving therein two or three pinches of hematoxylin crystals, until the solution is a bright cherry-red colour. The exact percentage of hematoxylin, Mallory states, is not of great importance, the main point being to stain deeply and decolorize slowly. The hematoxylin solution should be freshly prepared, and the surplus discarded. This method should be used by beginners, as it gives excellent results, and serves to obtain the experience necessary for the more difficult Heidenhain method.

Gauducheau's Iron-Hematoxylin-Eosin Stain. This is a very satisfactory modification of the Heidenhain method, and Dr. Gauducheau's illustrations with this paper were stained by it.

1. Distilled water.
2. Mordant forty-five minutes (or less) in a 2½% aqueous solution of iron-alum.
3. Rinse quickly in two changes of distilled water, dipping the preparations once in each change.
4. Stain in the same hematoxylin solution used in the Heidenhain method for fifteen minutes.
5. Decolorize in the mordant. This must be done very carefully, as the preparations quickly loss their colour.
6. Rinse in distilled water, and wash in tap water (preferably running), for fifteen minutes.
7. Stain in a 1/400 aqueous solution of eosin for twenty seconds.
8. Dehydrate in ascending alcohols, clear in xylol, and mount in acid-free balsam.

Seidelin's modification of Weigert's iron-hematoxylin stain, and

Rosenbusch's modification of the Heidenhain method are favourably spoken of. I have not used them personally, but I have seen excellent preparations made by Dr. Darling after the former method.

(*b*) *Mallory's Phosphotungstic Acid Hematin Stain.* This excellent stain has two advantages: it does not require a mordant, and it is ready for use in twenty-four hours. It is particularly valuable for demonstrating the chromatin of the nucleus, especially in separating the chromatin of the karyosome from the achromatinic substances, and also it shows very well the spindle-shaped arrangement of the chromatin during mitosis. The stain is prepared as follows:—

Hematin, preferably hematin ammonium	1 gramme.
10 % aqueous solution of phosphotungstic acid (Merck)	20 c.c.
Distilled water	100 c.c.

The stain is ripened by adding 5 c.c. of a ¼% aqueous solution of potassium permanganate, and can be used in twenty-four hours. For a full account of the stain see Mallory and Wright, 'Pathological Technique,' 1908 edition, pp. 277 and 344. The method given there is for tissues, and I have modified it somewhat for staining entamoebae.

1. Distilled water.
2. Stain in the phosphotungstic acid hematoxylin for twelve to twenty-four hours.
3. Rinse in distilled water.
4. Decolorize in a strong aqueous solution of orange G; I use a nearly saturated solution. The decolorization is not rapid, and requires about half an hour. Examine at intervals by washing the preparation in distilled water, and mounting in this, using a coverglass.
5. Wash thoroughly in two changes of distilled water, to remove all of the orange G.
6. Dehydrate as follows:—80 % alcohol, five minutes; two changes of 96 % alcohol, five minutes each; absolute alcohol, two changes, the first for five minutes, and the second for ten.
7. Clear in xylol, and mount in acid-free balsam.

When the preparation is made from viscid mucus or pus, the following addition to the technique is of service: —

1. Distilled water.
2. Permanganate of potassium, $\frac{1}{4}$% aqueous solution, five minutes.
3. Wash in water.
4. Oxalic acid, 5% aqueous solution, in which the preparations are to be moved until decolorized.
5. Wash thoroughly in several changes of water, preferably running tap water, to remove all traces of the oxalic acid.
6. Rinse in distilled water, and stain as directed above.

When very thin smears can be made from semi-liquid stools, instead of decolorizing in the orange G, the preparations may be taken directly from the stain into 96% alcohol. Transfer after ten minutes into fresh 96% alcohol, leave for five minutes, and take through absolute alcohol, two changes, xylol, to balsam for mounting. If the process is successful, the cytoplasmic detail will be very well preserved.

The only fault with the phosphotungstic acid stain is that in decolorization much of the cytoplasmic detail is lost. If some means can be found to correct this, the stain should become popular, on account of the faithful reproduction of many interesting phases of nuclear activity. The stain can be used many times, by filtering it back into the bottle, but after two or three months, it begins to over-stain too heavily, and should then be discarded.

Dr. S. T. Darling uses a very weak potassium permanganate solution for decolorization. When this is completed, he rinses the preparations in distilled water, dehydrates, clears in xylol, and mounts. This method emphasizes the nuclear structure, and, in his specimens from artificially infected cats, demonstrated the cycle of the karyosome very beautifully. It does not, unfortunately, give good cytoplasmic detail.

(*c*) *Alum Hematoxylin Stains.* These, in my experience, do not give good results in staining the entamoebae, as they are too diffuse to begin with, and become more so with age and use. This also seems to be the experience of many other workers, to judge from personal communications. The formulae for these stains can be found in any work on histology, and are given in detail in Mallory and Wright's 'Pathological Technique.'

Walker (1911) recommends very highly the use of aqueous alum hematoxylin, preferring it to the iron-hematoxylin methods, and states that it is more precise. This worker, as has been noted, failed to demonstrate the chromidia by its use, and although several excellent photographs accompany his contribution, these do not, in my opinion, show nuclear detail as clearly as after a properly used iron-hematoxylin stain. However, since Walker, by the use of this method, was the first to show the similarity between many phases of the *tetragena* and *histolytica* types, I give it here, as its simplicity, at least, has much to commend it.

Mallory and Wright give the preparation of the stain as follows:—

Hematoxylin crystals	1 gramme.
Saturated aqueous solution of ammonia alum	100 c.c.
Distilled water	300 c.c.
Thymol	a crystal.

Walker fixes five minutes in Zenker's fluid; washes in water until the fixing fluid is removed; stains five minutes in the hematoxylin solution; washes in distilled water; dehydrates; clears in oil origanum; and mounts in xylol balsam. He does not state if he hardens his preparations in alcohol before staining, which would seem necessary in order to procure good nuclear definition. Although I have tried this method repeatedly I have not had good results. But by prolonging the staining time to twelve hours, or over night, and later decolorizing in 70 % acid alcohol, I have sometimes obtained fairly good pictures. Such preparations, however, are very likely to fade quickly, while a good iron hematoxylin preparation will keep its colour for several years.

All hematoxylin stains, except the phosphotungstic acid hematin, dye the nucleus and cytoplasm in various shades of the same colour. As noted, some of them give excellent delineations of the nuclear structure, but none gives definite information as to included foreign bodies in the cytoplasm, such as erythrocytes in various stages of dissolution, bacteria, other cell nuclei, etc. Neither are the nuclear structures differentiated as after dry fixation and staining with Hastings and Giemsa, except for degrees of shading. In acute dysentery, very often body cells of different kinds are thrown out into the intestine, and some of these are phagocytic for erythrocytes. Frequently their cytoplasm is morphologically almost identical with that of the entamoebae, and their nuclei not seldom resemble certain phases of the nuclei of the entamoebae. When body cells and entamoebae are present in considerable numbers, hematoxylin staining has not infrequently led to confusion, and more than one author has described various phases of the entamoebic cycle from misinterpreted body cells. Counter-staining with cytoplasmic stains such as eosin, Bordeaux red, and acid fuchsin, unless very carefully done, often obscures nuclear detail without giving additional information.

The use of the Giemsa stain after wet fixation has given excellent results in the hands of experienced technicians, but this method requires much skill, and the use of absolutely pure reagents. Decolorization and dehydration are accomplished by acetone and mixtures of acetone and xylol, and good results are not easily procured.

(*d*) *Mann's Eosin-Methyl-blue Stain.* This stain has the advantage of giving a double polychrome stain with the use of one fluid. Although, as Mann (1902) states, the methyl-blue is constitutionally an acid dye, in many respects it acts as a colour base such as methylene-blue, while eosin also approaches in some respects the basic dyes. The method shows the chromatin segments of the nucleus in various shades from blue to red or orange, depending on the metabolic activities of the entamoebae, while the centriole shows in shades of orange. The karyosome also stains in various shades, and is very well demonstrated. Properly used, this stain produces excellent definition, and not only shows the functional activities of the nucleus, but also differentiates the various included bodies, and demonstrates very beautifully the phagocyted erythrocytes, both in the entamoebae and in the body cells. As noted in Part III, the study of the functional activities of the nuclei in the entamoebae has been overlooked by most authorities, and for the most part these activities have been interpreted as having a specific relation to the determination of species rather than as metabolic phenomena common to all species. This fact was pointed out to me by Dr. Mann, after he had made a study of my specimens.

Mann's stain, as far as I know, was first used for the study of the entamoebae by Elmassian (1909), who recommends it very highly. In a personal communication Dr. Elmassian has given me the technique used by him, which I present here, with notes of a few modifications. It is absolutely essential for good results to follow exactly this technique.

Elmassian advises fixation in Duboscq's fluid as essential to good results. I have, however, obtained good results after Schaudinn's and Zenker's fluids, and Mann speaks highly of the chrome-fixatives, especially Zenker's fluid. If the sublimate in Zenker's or Schaudinn's fluids is to be removed, alkalies such as the potassium iodide in Lugol's solution may be used, but alkalies should not be used to remove the iodine, which should be removed by alcohol. Mann states that preparations may be treated with the stain while still yellow with iodine, but it is perhaps preferable, in dealing with the entamoebae, to remove this with alcohol, as directed in the account of after treatment of fixation. If Duboscq's fluid is used as a fixative, Mann states that the preparations should be placed in absolute alcohol immediately after fixation, and this changed until the yellow colour is no longer discharged.

Mann's stain is made according to the following formula:—

1 % methyl-blue in distilled water	35 c.c.
1 % eosin in distilled water..................	45 c.c.
Distilled water	100 c.c.

After use the stain can be filtered back into the bottle, and used repeatedly.

The successful use of this stain depends largely on the decolorization. The decolorizing agent is a so-called 1 % mixture of potassium hydroxide in absolute alcohol, known as caustic alcohol. However, the caustic is not soluble in the alcohol, but rather traces of it are suspended therein. The caustic alcohol is prepared as follows:—1 gramme of solid potassium hydroxide, which must be thoroughly dry, is placed in a bottle that has a ground glass stopper, and 100 c.c. of absolute ethyl alcohol is added. The tightly-stoppered bottle is placed in an incubator at 37° C. overnight. In the morning, if a part of the caustic still remains, as it should do, the solution is filtered into another bottle, with a ground glass stopper, and tightly stoppered. If the caustic is entirely disintegrated, then a fresh mixture should be prepared, as the entirely disintegrated mixture forms too strong a reagent. The filtered mixture is the caustic alcohol used for decolorization. It is essential here, as throughout the technique, to use only pure absolute alcohol, as any trace of moisture in the alcohols, other than that carried over from the staining solution, will spoil the preparations.

1. The preparations are taken from the preserving alcohol to distilled water, in which they are washed in several changes thoroughly to remove all traces of alcohol.
2. Place in the stain for eight to twelve hours, not longer, otherwise the staining will be too deep, and decolorization will be difficult.
3. Rinse the preparations in distilled water for five seconds to remove the excess of stain and carry at once into absolute alcohol.
4. Dr. Elmassian advises two baths of this, leaving the preparations ten minutes in the first, and fifteen minutes in the second, to effect thorough dehydration. I use three baths of ten minutes each.
5. Transfer to the decolorizing solution, which is made by adding ten drops of the caustic alcohol to 60 c.c. of absolute alcohol. The proper amount of decolorization can be determined only by experience. As a general rule it may be said that decolorization is complete in fifteen to thirty minutes, depending on the strength of the caustic alcohol, which latter varies with the amount of caustic taken up when it was prepared. Also, a pink colour to the preparation is an indication that decolorization has proceeded far enough. With proper care, the preparation may be mounted in the absolute alcohol, covered with a coverglass, and examined with a high dry power or a water or oil immersion lens. If either of the last is used, the coverglass must be removed before placing the preparation back in the absolute alcohol. The preparations should be moved occasionally to assist in decolorization.

6. After decolorization is completed, wash carefully in *fresh* absolute alcohol to remove all traces of the caustic alcohol. Dr. Elmassian dips each preparation twenty times.
7. Dip hastily in distilled water to remove excess of eosin.
8. Take to fresh absolute alcohol, dip the preparation twenty times, and transfer to a second bath of fresh absolute alcohol for twenty minutes.
9. Clear in xylol, and mount in acid-free balsam. Any trace of acid in the mounting medium will quickly decolorize the preparation.

Instead of going from absolute alcohol to distilled water in step 7, the slides may be carried directly into xylol and mounted. Sometimes this washing in distilled water will over-decolorize the preparation, and also will destroy the delicate colour reactions of the nuclear chromatin. If one is making several preparations, it is perhaps best to follow the full technique with one, and take another through to mounting without washing. The preparations can then be compared, and that method which seems preferable used for the rest of the preparations. I have seen a preparation of Dr. Elmassian's stained by the full technique as given above, in which the definition of the nuclear contents was as sharp and clear as could be desired.

After the preparations have been mounted, they should be examined at once with the oil immersion. If they are over-decolorized, they may be placed in xylol over night, to remove the balsam; in absolute alcohol for an hour to remove the xylol; carried down to distilled water, and re-stained. If they are not sufficiently decolorized, they should be carried from the absolute alcohol used to remove the xylol, through a bath of fresh absolute alcohol to the decolorizing alcohol, and treated therein.

BIBLIOGRAPHY

Note.—A complete bibliography of the parasitic amoebae (entamoebae) has been compiled by Mr. Albert Hassall, Assistant Zoologist, United States Department of Agriculture, Washington, D.C.

Those references which I could not consult personally I have taken from the list of Mr. Hassall, to whom I wish to take this opportunity to express my obligation. Mr. Hassall's list is entitled 'Bibliography of Parasitic Amoebae,' and is published in the Transactions of the Fifteenth International Congress on Hygiene and Demography, Vol. II, Part 1, Proceedings of Section I, Hygienic Microbiology and Parasitology, pp. 198-286. Washington, Government Printing Office, 1913.

Brown, W. Carnegie (1910). Amoebic or Tropical Dysentery, London.

Calandruccio, Salvatore (1889-1890). Animali parassiti dell' uomo in Sicilia. Atti. Acad. Gioenia di sc. nat. in Catania, an. 66, 4 s., Vol. 2, pp. 95-135.

Calkins, Gary N. (1909). Protozoology, New York.

Casagrandi, Oddo G. V., and Barbagallo-Rapisardi, Pietro (1895). Ricerche biologiche e cliniche sull' *Amoeba coli* (Lösch). Nota preliminare. Boll. Accad. Gioenia di sc. nat. in Catania, n.s. (39), gennaio, pp. 4-14.

——— (1895*b*). Ricerche biologiche e cliniche sull' *Amoeba coli* (Lösch). Seconda ed ultima nota preliminare. Ibid (41), nov., pp. 7-19.

——— (1895*c*). Sull' *Amoeba coli* (Lösch), ricerche biologiche e cliniche. Catania.

——— (1897). *Entamoeba hominis s. Amoeba coli* (Lösch). Ann. d'ig. sper., Roma, n.s., Vol. 7 (1), pp. 103-166.

Celli, Angelo, and Fiocca, R. (1894*a*). Contributo alle conoscenze sulla vita delle amebe. 1a. Nota preventiva. Riforma med., Napoli, an. 10, Vol. 1 (68), 22 mar., pp. 806-808.

——— (1894*b*). Communicazione preventiva sulla classificazione delle amebe e di alcune specie finora cultivate. Ibid., Vol. 3, 13 agosto, pp. 434-441.

——— (1894*c*). Beiträge zur Amöebenforschung. Erste vorlaüfige Mitteilung. (Transl. of 1894*a*.) Centralb. f. Bakteriol. (ect), Jena, Vol. 15, April 7, pp. 470-473.

——— (1894*d*). Idem. Zweite vorläufige Mitteilung. Uber die Klassifikation der Amöeben und einige gezüchtete Species. (Transl. of 1894*b*.) Centralb. f. Bakteriol. (ect), Jena, Vol. 16, pp. 329-339.

——— (1895*a*). Intorno alla biologia delle amebe. Ann. Ist. d'ig. sper. Univ. di Roma, n.s., Vol. 5, pp. 177-213.

——— (1895*b*). Idem. Bull. r. Accad. med. di Roma, Vol. 21, pp. 285-324.

Chatton, Édouard, and Brodsky, A. (1909). Le parasitisme d'une chytridinée du genre *Sphaerita* Dangeard chez Amoeba *limax* Dujard. Arch. f. Protistenk., Jena, Bd. 17, H. 1, pp. 1 18.

Chatton, Édouard, and Lalung-Bonnaire, P. (1912). Amibe limax (Vahlkampfia, n.gen.) dans l'intestin humain. Son importance pour l'interprétation des amibes de culture. Bull. Soc. de path. exot., Paris, Vol. 5, pp. 135-143.

Councilman, William T., and Lafleur, Henri A. (1891). Amoebic Dysentery. Johns Hopkins Hosp. Reports, Baltimore, Vol. 2, Dec., pp. 393-548.

CRAIG, CHAS. F. (1905). Observations upon amebas infecting the human intestine, with a description of two species, *Entamoeba coli* and *Entamoeba dysenteriae*. American Med., Philadelphia, Vol. 9, no. 21, May 27, pp. 854-861; no. 22, June 3, pp. 897-903; no. 23, June 10, pp. 936-942.

——— (1908). Studies upon the amoebae in the intestine of man. Journ. Infec. Dis., Chicago, Vol. 5, no. 3, June 4, pp. 324-377.

——— (1911). The parasitic amoebae of man. Philadelphia.

——— (1913). The identity of *Entamoeba histolytica* and *Entamoeba tetragena*, with observations upon the morphology and life-cycle of *Entamoeba histolytica*. Journ. Infec. Dis., Chicago, Vol. 13, no. 1, July, p. 1.

DANGEARD, P. A. (1894-95). Parasites du noyau et du protoplasma. Le Botaniste, Fasc. 6, Potiers.

DARLING, S. T. (1912*a*). Report Dept. Sanit., Isthmian Canal Com. Mt. Hope, Canal Zone, March, 1912, p. 42.

——— (1912*b*). The examination of stools for cysts of *Entamoeba tetragena*. Journ. Trop. Med. and Hyg., Vol. 15, no. 17, Sept. 2, pp. 257-259.

——— (1912*c*). The identification of the pathogenic entamoebae of Panama. Trans. 15th International Congress Hyg. and Dem., Washington, Vol. 2, part 1, pp. 168-174.

——— (1913*a*). Observations on the cysts of *Entamoeba tetragena*. Arch. Int. Med. Chicago, Vol. 11, p. 1.

——— (1913*b*). The rectal inoculation of kittens as an aid in determining the identity of pathogenic entamoebae. Bull. Soc. Path. Ex., Paris, Vol. 6, no. 3.

——— (1913*c*). Budding and other changes described by Schaudinn for *Entamoeba histolytica*, seen in a race of *Entamoeba tetragena*. Journ. Amer. Med. Assn., Chicago, Vol. 60, p. 1220, April 19.

——— (1913*d*). Budding and other forms in trophozoites of *Entamoeba tetragena*. Arch. Int. Med., Chicago, Vol. 11, p. 445, May.

DOFLEIN, F. (1909). Lehrbuch der Protozoenkunde, Jena.

ELMASSIAN, M. (1909*a*). Sur *l'Amoeba blattae*. Morphologie génération. Arch. f. Protistk., Jena, Bd. 16, H. 2, pp. 143-163.

——— (1909*b*). Sur une nouvelle espèce amibienne chez l'homme, *Entamoeba minuta*, n.sp. Centralbl. f. Bakteriol., Jena, 1 Abt., Vol. 52, no. 3, November 19, Orig., pp. 335-351. The plates are in the following number.

FANTHAM, H. B. (1911). On the Amoebae parasitic in the Human Intestine, with Remarks on the Life-Cycle of *Entamoeba coli* in Cultures. Annals Trop. Med. & Parasitol., V, pp. 111-123.

GRASSI, GIOVANNI B. (1879). Dei protozoi parassiti e specialmente di quelli che sono nell'uomo. Gazz. Med. ital. lomb., Milano, Vol. 39, 8 s., Vol. 1 (45), Nov. 8th, pp. 445-448.

——— (881). Contribuzione allo studio delle amibe. R. Ist. Lomb. di sc. e lett. Rendic., 2 s., Vol. 14, pp. 353-356.

——— (1882). Intorno ad alcuni protosti endoparassitici, ect. Atti Soc. ital. di. sc. nat., Milano, Vol. 24, nos. 2-3, Feb., pp. 135-224.

GAUDUCHEAU, A. (1912*a*). Sur quelques conditions biologiques que déterminent le développement et la forme des amibes. Bull. Soc. Medico-Chirurgicale, Hanoi-Hapong, May.

——— (1912*b*). Recherches sur la multiplication des Entamibes. Trans. Far Eastern Assn. Trop. Med., Second Biennial Congress, Hong Kong.

HARTMANN, MAX. (1908). Eine neue Dysenterieamöebe, *Entamoeba tetragena* (Viereck) syn. *Entamoeba africana* (Hartmann). Beiheft z. Arch. f. Schiffs- u. Tropen-Hyg., Leipzig, Bd. 12, H. 5, August, pp. 117-127.

——— (1909). Untersuchungen über parasitische Amöben. 1. *Entamoeba histolytica*, Schaudinn. Arch. f. Protistk., Jena, Bd. 18, H. 2, pp. 207-220.

——— (1912). Untersuchungen über parasitische Amöben. 2. *Entamoeba tetragena*, Viereck. Ibid., Bd. 24, H. 3, pp. 163-181.

HARTMANN, M., and VON PROWAZEK, ST. (1907). Blepharoplast, Caryosom, und Centrosome. Arch. f. Protistk., Bd. 10, pp. 306-335.

Hartmann, M., and Whitmore, E. (1912). Untersuchungen über parasitische Amöben. 3. *Entamoeba coli*, Lösch em. Schaudinn. Arch. f. Protistenk., Bd. 24, H. 3, pp. 182-194.

James, William M. (1912*a*). The clinical identification of the Entamoebae. Proc. Canal Zone Med. Assn., Mt. Hope, C.Z., Vol. 4, Part 2.

——— (1912*b*). Infection with *Entamoeba tetragena*. Ibid., Vol. 5, Part 1. Also in New York Med. Journ., Vol. 97, p. 702, 1913.

Jürgens (or Juergens), R. (1902). Zur Kentniss der Darm-Amöben und der Amöben-Enteritis. Veroffentl. a. d. Geb. d. Militarsanitatswesen, Berlin, Heft 20, pp. 110-160.

——— (1907). Die Amöben-enteritis und ihre Beziehungen zur Dysenterie, Ztschr. f. exper. Path. u. Ther., Berlin, Bd. 4, H. 3, December 21, pp. 769816.

Kartulis, Stephan (1885). Über Riesen-Amöben bei chronischer Darmentzündung der Aegypter. Arch. f. Path. Anat., Berlin, Bd. 99, F. 9, Vol. 9 (1), Jan. 2, pp. 145-147.

——— (1887). Zur Aetiologie der Leberabscesse. Lebende Dysenterie-Amöben im Eiter der dysenterichen Leberabscesse. Centralbl. f. Bakteriol., Bd. 2, H. 25, pp. 745-748.

——— (1889). Uber tropische Leberabscesse und ihre Verhaltnisse zur Dysenterie. Arch. f. path. Anat. Berlin, Bd. 118, F. 11, Vol. 8 (1), Oct. 1, pp. 97-121.

——— (1890). Uber weitere Verbreitungsgebiete der Dysenterie-Amöben. Centralbl. f. Bakteriol., Bd. 7, H. 2, Jan. 4, pp. 54-55.

——— (1891). Einiges über die Pathogenese der Dysenterieamöben. Ibid., Bd. 9, H. 11, March 21, pp. 365-372.

——— (1906). Die Amöbendysenterie. Handb. d. path. Mikroorganism, Jena. 1. Ergänzungsbd, pp. 347-384.

Koidzumi, M. (1909). On a new parasitic *Amoeba*, *Entamoeba nipponica*, etc. Centralbl. f. Bakteriol., I. Abt. Bd. 51, H. 6, October 9, Orig., pp. 650-654.

Kruse, Walther, and Pasquale, Alessandro (1893). Eine Expedition nach Egypten zum Studium der Dysenterie und des Leberabscesses. Deutsche med. Wchnschr., Leipzig and Berlin, Bd. 19, H. 15, April 13, p. 354; H. 16, April 20, pp. 378-379

——— (1894). Untersuchungen über Dysenterie und Leberabscess. Ztschr. f. Hyg. u. Infectionskrankh., Leipzig, Bd. 16, pp. 1-148.

Lesage, A. (1905). Culture de l'amibe de la dysenterie des pays chauds. Ann. de l'Inst. Pasteur, Paris, Vol. 19, No. 1, Jan. 25, pp. 9-16.

——— (1908). Note sur les entamibes dans la dysenterie amibienne des pays chauds. Bull. Soc. de path. exot., Paris, Vol. 5, no. 2, Feb. 12, pp. 104-111.

Mercier, L. (1909). Le cycle évolutif *d'Amoeba blattae*, Bütschli. (Note préliminaire.) Arch. f. Protistenk., Bd. 16, H. 2, pp. 164-168.

——— (1910). Contribution a l'etude de l'amibe de la blatte (*Entamoeba blattae*, Bütschli). Ibid., Bd. 20, H. 2, pp. 143-175.

Minchin, E. A. (1912). Introduction to the study of Protozoology, London.

Noc, F. (1909). Recherches sur la dysenterie amibienne en Cochinchine. Ann. de l'Inst. Pasteur, Paris, Vol. 5, no. 3, Mar. 25, pp. 177-204.

von Prowazek, St. (1911). Beitrag zur *Entamoeba*-Frage. Arch f. Protistenk., Bd. 22, H. 3, pp. 345-350.

——— (1912*a*). *Entamoeba*. Ibid., Bd. 25, H. 2, pp. 273-274.

——— (1912*b*). Weiterer Beitrag zur Kentniss der Entamöben. Ibid., Bd. 26, H. 2, pp. 241-249.

Quincke, H., and Roos, Ernst (1893). Uber Amöben-Enteritis. Berl. klin. Wchnschr., Bd. 30, H. 45, Nov. 6, pp. 1089-1094.

Ruge, Reinhold (1906). Amöbenruhr. Handbuch d. Tropenkrankh. (Mense), Leipzig, Bd. 3, pp. 1-21.

Schaudinn, Fritz (1903). Untersuchungen über die Fortpflanzung einiger Rhizopoden. Arb. a. d. k. Gsndhtsamte, Berlin, Bd. 19, H. 3, pp. 547-576.

Schuberg, August (1893). Die parasitischen Amöeben des menschlichen Darmes (etc.). Centralbl. f. Bakteriol., Bd. 13, H. 18-19, May 4, pp. 598-609; H. 20, May 17, pp. 654-665; H. 21-22, May 29, pp. 701-714.

Viereck, H. (1907). Studien über die in den Tropen erworbene Dysenterie (Dysenterie tropical). Arch. f. Schiffs- u. Tropen-Hyg., Bd. 11, Bei. 1.

Walker, Ernest Linwood (1911). A comparative study of the amoebae in the Manila water supply, in the intestinal tract of healthy persons, and in amoebic dysentery. Philippine Jour. Sc. Manila, Sec. B, Vol. 6, no. 4, Oct., pp. 259-279.

Walker & Sellards (1913). 'Experimental Entamoebic Dysentery.' Philippine Jour. Sc., B, VII, p. 253.

Wenyon, C. M. (1907). Observations on the Protozoa in the intestine of mice. Arch. f. Protistenk. Suppl. 1, Festbd. z. 25 jahr. Prof.-Jubil. Richard Hertwig, pp. 169-201.

——— (1912). Experimental amoebic dysentery and liver-abscess in cats. Journ. London School of Trop. Med., Vol. 2, no. 1, Dec. pp. 27-34.

Werner, Heinrich (1908). Studien über pathogene Amöben. Beihefte z. Arch. f. Schiffs- u. Tropen-Hyg., Bd. 12, Bei. 11, pp. 5-18.

——— (1911). *Entamoeba coli.* Handb. d. path. Protozoen (v. Prowazek), Leipzig, 1 Lief., pp. 67-77.

Whitmore, Eugene (1911*a*). Parasitäre und freilebende Amöben aus Manila und ihre Beziehungen zur dysenterie. Arch. f. Protistenk., Bd. 23, H. 1-2, pp. 71-80.

——— (1911*b*). Vorlaüfige Bemerkungen über Amöben aus Manila und Saigon. Centralbl. f. Bakteriol., 1 Abt., Bd. 58, H. 3, April 1, Orig., pp. 234-235.

——— (1913). Free living and parasitic *Amoebae* and their relation to dysentery. Amer. Jour. Trop. Dis. and Prevent. Med., New Orleans, Vol. 1, no. 3, Sept., p. 197.

EXPLANATION OF PLATES

All Figures drawn by me were made with a Zeiss I.B. stand, and the 2 mm. apochromatic objective, 1·40 N.A., with a No. 12 compensating ocular. They are all drawn to the same scale, with the aid of the Bausch and Lomb Abbé drawing apparatus, and the Bausch and Lomb drawing table. The paper was placed at the level of shoulder of the objective. The initial magnification is about 1,500, and the projected magnification about 1,950.

Unless otherwise indicated, all drawings were made from preparations fixed in Schaudinn's fluid, plus 3 % glacial acetic acid, and stained by Heidenhain's iron-hematoxylin method, using a 3½% solution of iron and ammonium alum as a mordant and decolorizing agent. All numbers given are inclusive.

PLATE XII

Figs. 1-16. *Histolytica* type. Type A.

Figs. 1-3. Large vegetative organisms, with phagocyted erythrocytes.

Fig. 4. Vegetative organism, showing structure of cytoplasm during movement.

Figs. 5-9. Organisms in the period following subsidence of acute symptoms, while the stools are yet watery and contain mucus, but not blood and pus. In the pure *histolytica* type I was unable to follow these organisms past this stage, but in the Type C infections it preceded cyst formation. The variations in nuclear appearance are due more probably to the functional state of the organisms than to errors in technique.

Fig. 5. Small organism without chromidia.

Figs. 6-7. Chromidia formation. In Fig. 7 bacteria are also seen in the upper left section of the entamoeba.

Figs. 8-9. Variations in nuclear staining.

Figs. 10-16. Moist chamber preparations, showing the so-called 'bud' formation described by Schaudinn.

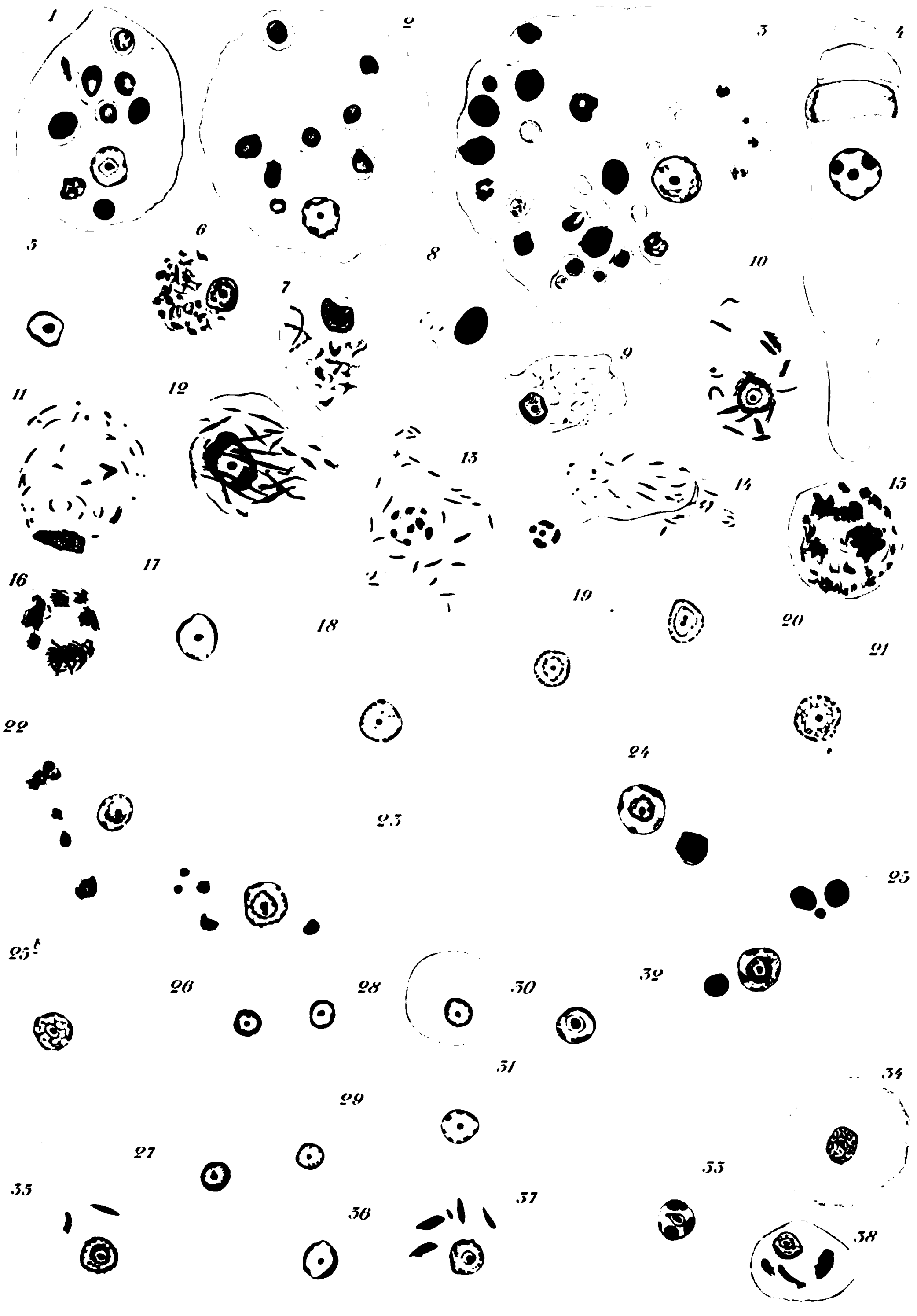
Fig. 10. Beginning so-called chromidia formation by the nucleus.

Fig. 11. 'Distortion' and 'degeneration' of nucleus following distribution of chromidia to cytoplasm.

Fig. 12. Swollen nucleus, showing abundant collection of peripheral chromatin, and distribution (?) of this in large rods to the cytoplasm.

Figs. 13-14. 'Budding' organisms, showing distribution (?) of chromidia to the 'buds.' Fig. 14 shows very plainly the nucleus in one of the 'buds' of ectoplasm, the so-called 'extrusion' of the nucleus.

Figs. 15-16. 'Degeneration' of the nucleus, with apparent conversion (?) of most of it into chromidia.


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
25t
26
27
28
29
30
31
32
33
34
35
36
37
38

[illegible]

Figs. 17-21. Variations of nuclear structure in the intermediate type, Type C. The karyosome, though plainly evident, does not show as well as in Type B, the *tetragena* type. The cycle of the entamoebae in infections with this type originated in the large vegetative forms shown in Figs. 17-21, passed through the phases represented in Figs. 5-9, and terminated in cyst formation as shown in Figs. 41-56. In one case through Figs. 26-31 also.

Figs. 22-56. Cycle of the *tetragena* type, Type B.

Figs. 22-25*b*. Cycle of the karyosome in the large vegetative organisms.

Fig. 22. Differentiation of the centriole and karyosome.

Fig. 23. Formation of the karyosome margin, and beginning collection of chromatin under the nuclear membrane.

Fig. 24. Clear zone around the centriole well defined, collection of chromatin at the karyosome margin, and further collection of chromatin under the nuclear membrane.

Fig. 25*a*. The chromatin is collected mostly at the karyosome margin and under the nuclear membrane.

Fig. 25*b*. The chromatin has passed out over the achromatinic network to the nuclear periphery, leaving only the centriole and the karyosome margin. According to Hartmann (1912), the cycle will now renew itself, if the entamoeba does not divide, by a swelling of the centriole to form a new karyosome.

Figs. 26-31. *Minuta* type. Small generation preceding cyst formation, observed in one Type B infection, and followed by the phases represented by Figs. 32-40. In the other infections with this type, the cycle passed from the phases represented by Figs. 22-25*b*, through the precystic generation shown in Figs. 32-40, to cyst formation. Figs. 26-31 resemble very closely the *minuta* type of Elmassian, and he has suggested that perhaps a double infection was present.

PLATE XIII

Figs. 41-45. Cyst formation. These figures show the only cyst formation observed by me, following infections with any of the pathogenic species.

Fig. 41. One-nucleated cyst, taken from a stool 24 hours old.

Fig. 42. The same, with small vacuole.

Fig. 43. Beginning formation of the 'spindle.'

Figs. 44-46. Further phases of primary division of the nucleus. Figs. 44 and 46 show side views of the spindle, and Fig. 45 shows a polar view. Fig. 45 is also very similar to those described by various authorities as the result of conjugation of the nuclei after reduction.

Fig. 47. Two-nucleated cyst, resting stage, as shown by well-defined centrioles.

Fig. 48. Two-nucleated cyst, beginning nuclear division.

Fig. 49. Two-nucleated cyst, 'spindle' forms of the nuclei.

Fig. 50. Three-nucleated cyst, one nucleus not divided, the two others in the end stage. This phase was also observed when a vacuole was present. This suggests the so-called 'reduction' nuclei.

Fig. 51. Three-nucleated cyst, in which the nuclei are apparently all of the same size and stage. No sign of a fourth nucleus could be made out.

Figs. 52-55. Four-nucleated cysts, end stage.

Fig. 56. Very rare four-nucleated form, which from its size and structure is apparently a schizont. This form has been pictured by Viereck (1907), and by Darling (1913*a*), the latter describes his as 'Trophozoite with primary division of the nucleus into four nuclei. This form is rarely seen, and is believed to be a schizont.'

Figs. 57-94. Cycle of *E. coli*.

Figs. 57-59. Vegetative phases of *E. coli*.

Figs. 57-58. Usual type of vegetative organisms.

Figs. 59-61. Apparent beginning division of karyosome, or centriole. As explained in Part 2, I am by no means convinced that the central circular mass in the nucleus of *E. coli* is a true karyosome and is not an enlarged centriole, but I believe it is better to follow the present nomenclature, and call it the karyosome.

Fig. 62. Unusual type of vegetative organism, without a nucleus.

Fig. 63. Vegetative organism, which has phagocyted a number of foreign bodies, probably yeasts. The nucleus shows a divided karyosome.

Fig. 64. Nucleus, showing a tri-polar karyosome.

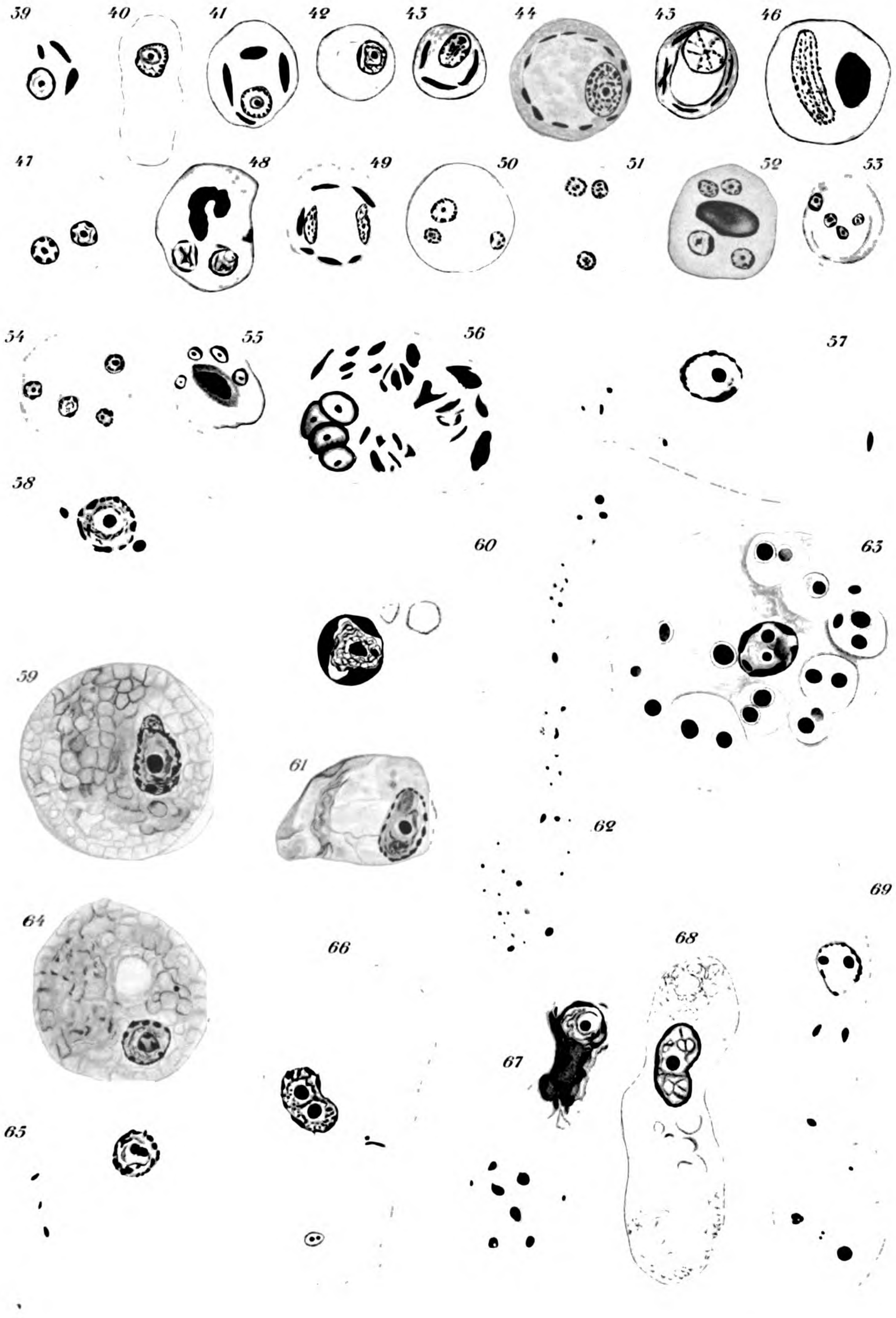
Fig. 65. Nucleus, showing a bi-polar karyosome.

Fig. 66. Apparent division of entire nucleus, nearly completed.

Fig. 67. So-called 'extrusion' of the nucleus. The dark staining substance just posterior to the nucleus appears to be cytoplasm, and not included material.

Fig. 68. Apparent division phase of nucleus. In this instance the appearance may be due to a mechanical constriction.

Fig. 69. Elongation of the organism, with central constriction.



Firenze, Lit. A. Ruffoni, Piazza S. Croce 20

PLATE XIV

Figs. 70-75. Pre-cystic phases of *E. coli.*

Figs. 70-72. These show a condition very similar to that pictured in Figs. 5-9 for the pre-cystic stages of *histolytica.* The deeply-stained masses could not be distinguished either morphologically or by staining reactions from so-called chromidia, but may be inclusions.

Fig. 73. Apparent beginning extrusion of cell contents from a pre-cystic form. The interpretation of such figures is doubtful, but the structure of the cytoplasm shows a departure from that of the vegetative organisms.

Fig. 74. Pre-cystic organism.

Fig. 75. This organism appears to have a rupture of the limiting membrane. This may be due to mechanical causes, or to extrusion of contents.

Figs. 76-94. Cyst formation in *E. coli.*

Fig. 76. One-nucleated cyst, resting stage. From a 24 hours' old stool.

Figs. 77-79. Phases in primary division of the nucleus.

Fig. 80. Two-nucleated, resting stage.

Figs. 81-82. These may represent the forms described by Schaudinn as resulting from the dissolution of the nuclei following primary division, although I believe that in Fig. 81 the nucleus is only partly hidden, and in Fig. 82 entirely hidden by the dark mass, which latter appears to be contained in a vacuole. These were the only two cyst forms in which I could not distinguish phases of nuclear division, or resting stages.

Fig. 83. Two-nucleated cyst, with central chromatin loosely arranged.

Fig. 84. Two-nucleated cyst, with central chromatin compactly arranged.

Fig. 85. Two-nucleated cyst, side and polar views of nuclear division.

Fig. 86. Two-nucleated cyst, with light staining nuclei.

Fig. 87. Two-nucleated cyst, with dark staining nuclei.

Fig. 88. Four-nucleated cyst, resting stage.

Fig. 89. Four-nucleated cyst, nuclei in division.

Fig. 90. Eight-nucleated cyst, with chromidia, granular type.

Fig. 91. Cyst, showing six nuclei in end stage and the seventh in division, granular type.

Fig. 92. Eight-nucleated cyst, granular type, without chromidia.

Fig. 93. Large eight-nucleated cyst, alveolar type.

Fig. 94. Large fifteen-nucleated cyst, alveolar type.

Figs. 95-99. Series showing so-called transition between free nucleus and small entamoeba.

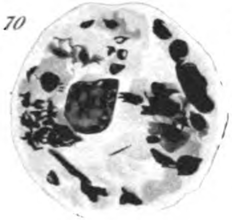
Fig. 95. Free nucleus. Note formation of ' plastin ' around the karyosome.

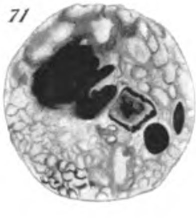
Fig. 96. Nucleus, with small amount of cytoplasm.

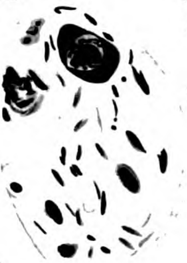
Fig. 97. Small entamoeba, with large nucleus.

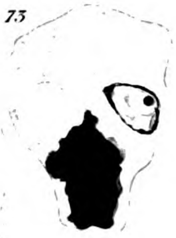
Fig. 98. Small entamoeba, with well-defined ' spindle ' of karyosome.

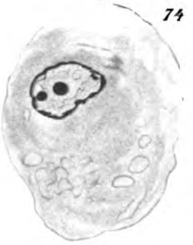
Fig. 99. Small entamoeba, with two large karyosomes (?) in nucleus.

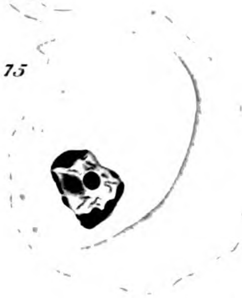
70


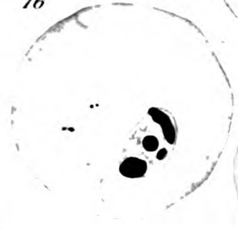
71


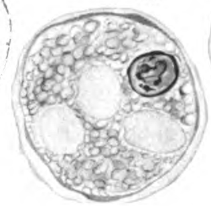
72


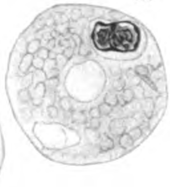
73


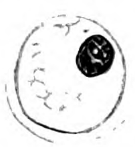
74


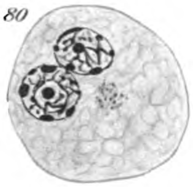
75


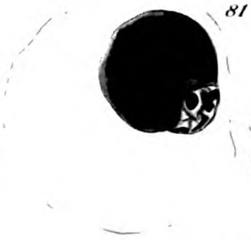
76


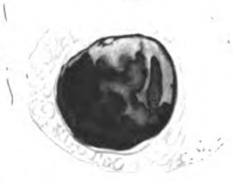
77


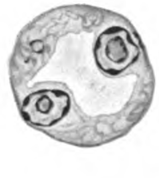
78


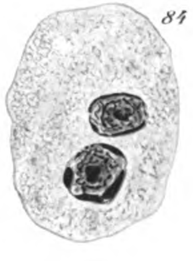
79


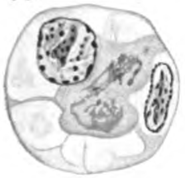
80


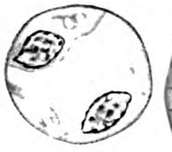
81


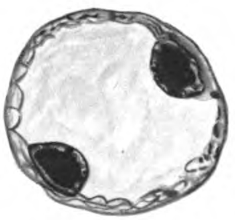
82


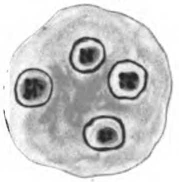
83


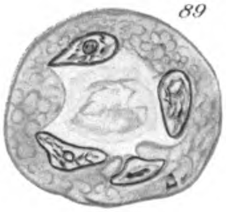
84


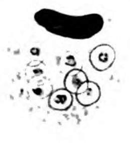
85


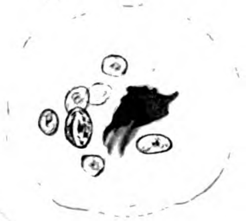
86


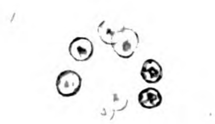
87


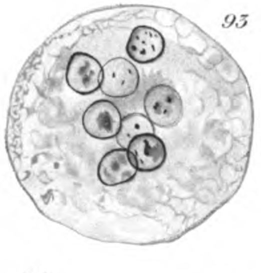
88


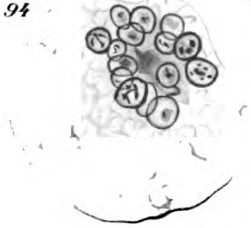
89


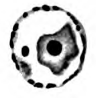
90


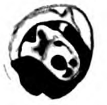
91


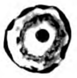
92


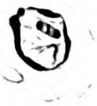
93


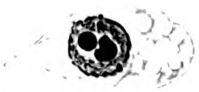
94


95


96


97


98


99


Firenze Lit. A. Ruffoni, Piazza S. Croce 20

PLATE XV

Figs. 100-116. Drawn by Dr. M. Elmassian. Objective, 2 mm. apochromatic, No. 12 eyepiece. Paper at the level of the stage of the microscope. Dimensions given below with each figure.[*] These drawings were made from specimens sent by me to Dr. Elmassian.

Figs. 100-104. Vegetative organisms of the *histolytica* type. Note the differentiation between the minute centriole and the small karyosome. Fig. 103 shows included erythrocytes.

Figs. 105-106. Two-nucleated vegetative organisms, *histolytica* type.

Fig. 107. Small organism, *histolytica* type. Dr. Elmassian suggests that this may show beginning cyst formation.

Fig. 108. Vegetative organism, *tetragena* type, showing well-defined achromatic network. The karyosome is represented by the collection of small chromatin granules surrounding the centriole.

Fig. 109. One-nucleated cyst, *tetragena* type.

Fig. 110. Four-nucleated cyst, *tetragena* type.

Figs. 111-113. ***Entamoeba minuta.***

Fig. 111. Vegetative organism.

Fig. 112. One-nucleated cyst.

Fig. 113. Four-nucleated cyst.

A comparison of these figures with those drawn by Dr. Elmassian for *E. tetragena*, shows the difference in size and nuclear structure pointed out by him in his paper (1909).

Figs. 114-116. Free living amoebae of the *limax* type, from the human intestinal tract.

Figs. 114-115. Vegetative forms.

Fig. 116. Cyst.

Fig. 100. Diameter (D.) 22μ, Nucleus (N.) 6μ.

Fig. 101. D. 20μ, N. 5μ. Fig. 102. D. 24μ, N. 6μ. Fig. 103. D. 26μ, N. 5μ. Fig. 104. D. 18μ, N. 5μ. Fig. 105. D. 20μ, N. 4μ. Fig. 106. D. 18μ, N. 5μ. Fig. 107. D. 12μ, N. 6μ. Fig. 108. D. 28μ, N. 7μ. Fig. 109. D. 16μ, N. 6μ. Fig. 110. D. 16μ, N. 4μ. Fig. 111. D. 14μ, N. 4μ. Fig. 112. D. 12μ, N. 4μ. Fig. 113. D. 12μ, N. 2μ. Fig. 114. D. 18μ, N. 5μ. Fig. 115. D. 18μ, N. 5μ. Fig. 116. D. 12μ, N. 5μ.

Figs. 117-119. Free living amoebae from the human intestinal tract.

Figs. 117-118. Vegetative organisms.

Fig. 119. Cyst.

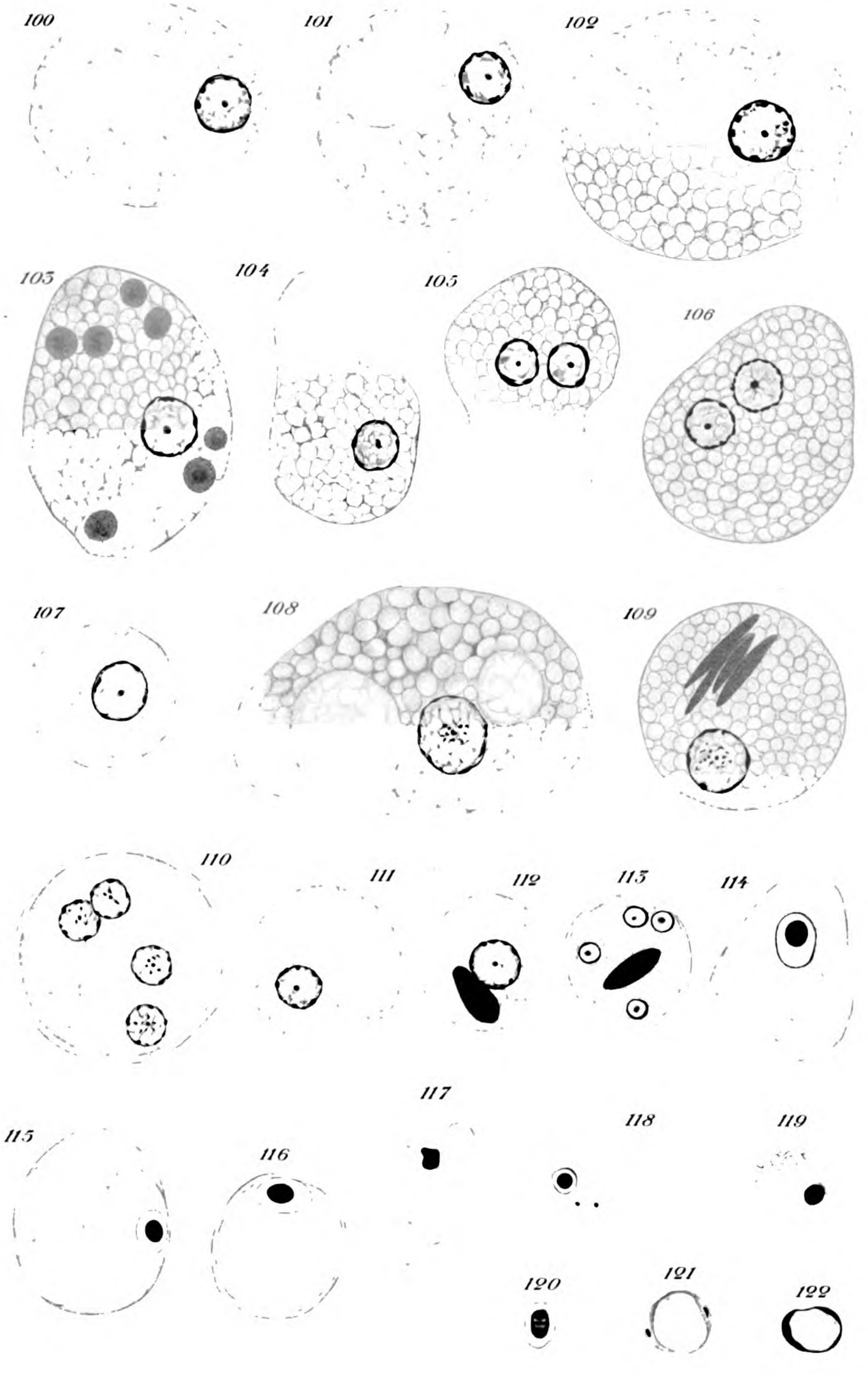
Fig. 120. Nucleus in division.

Figs. 121-122. Cysts of *Trichomonas intestinalis.*

---

[*] Dimensions of figures drawn by Dr. Elmassian.

PLATE XV



Firenze, Lit. A. Ruffoni, Piazza S. Croce 20

PLATE XVI

Fig. 123. Drawn by Dr. A. Gauducheau. Magnification, about 800. Fixed in Gauducheau's sublimate-chrome-acetic-acid solution, and stained with eosin-iron-hematoxylin. (See Part IV). Dr. Gauducheau states that this is the common form of entamoeba met with in the amoebic dysentery of Indo-China. As can be seen, the drawings correspond to the *histolytica* type of nucleus, and show very slight indications of karyosome formation.

Figs. 124-142. Dry fixed preparations, stained with Hastings' and Giemsa's stains, and decolourised in alcohol, 60%, to which has been added about 1% aqueous solution of ammonia, Darling's method.

Figs. 124-136. Phases of the cycle of *Ent. histolytica.*

Figs. 124-125. Vegetative organisms, *histolytica* type. The darker ectoplasm is shown at the upper right margin in Fig. 125.

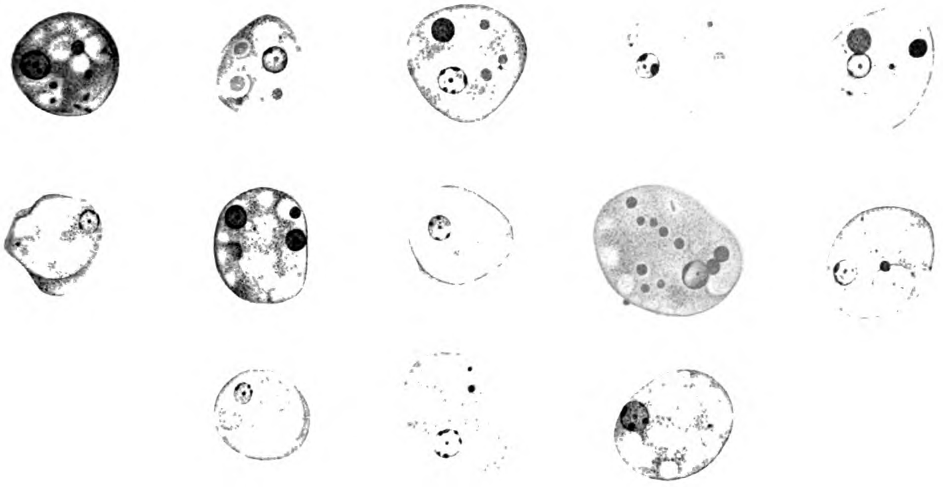
Fig. 126. *Tetragena* type, vegetative organism.

Figs. 127-131. Pre-cystic generation. Figs. 127 and 128 show chromidia in deep blue masses.

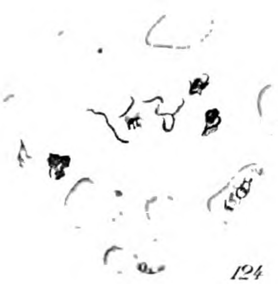
Fig. 129. The large amount of substance staining red in this organism does not stain by iron-hematoxylin, and is not chromidia. This preparation was made from the same stool as that from which the preparation of Figs. 26-31 was also made, and no chromidia formation was seen.

Figs. 130-131. Degenerating entamoebae.

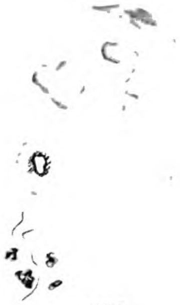
Figs. 132-136. Cyst formation. The chromidia stain a very deep blue. Fig. 132 has an initial magnification of 500.



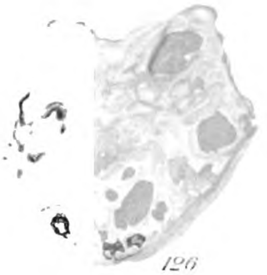
123



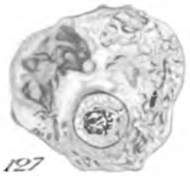
124



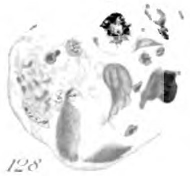
125



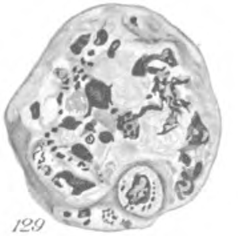
126



127

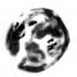


128



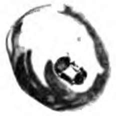
129

130

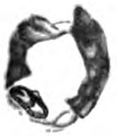


132

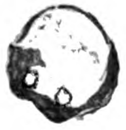
131



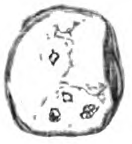
133



134



135



136

[illegible]

PLATE XVII

Figs. 137-142. Phases of the cycle of *Entamoeba coli.* Dry Fixation and Hastings' and Giemsa's stains.

Figs. 137-140 and Fig. 144. Vegetative entamoebae.

Fig. 137*a*. Shows two *Trichomonas* phagocyted, with stool débris. Fig. 137*b* shows a free *Trichomonas.*

Fig. 138. Very large vegetative entamoeba, showing the typical structure of the *coli* nucleus after this method of staining.

Fig. 139. Shows numerous phagocyted bacteria and other contained matter, much of which does not stain by iron hematoxylin. There is a very well-defined ectoplasm, staining a light blue.

Figs. 140 and 144. Various aspects of the vegetative phase.

Fig. 141. Pre-cystic organism.

Fig. 142. Cyst formation. *Coli* cysts do not show, except very rarely, the internal structure, after Hastings' and Giemsa's stains, but show it very well after wet-fixation and Mann's stain (Fig. 143).

Fig. 143. Two-nucleated *coli* cyst, with vacuole. Note the staining reaction of the so-called chromidia.

Figs. 145-156. Fixation with Schaudinn's fluid and stained by Mann's method.

Figs. 145-146. Vegetative forms of the *histolytica* type.

Figs. 147-148. Vegetative forms of the *tetragena* type.

Fig. 149. Free nucleus of *E. coli.*

Fig. 150. Possibly a stage of *T. intestinalis.*

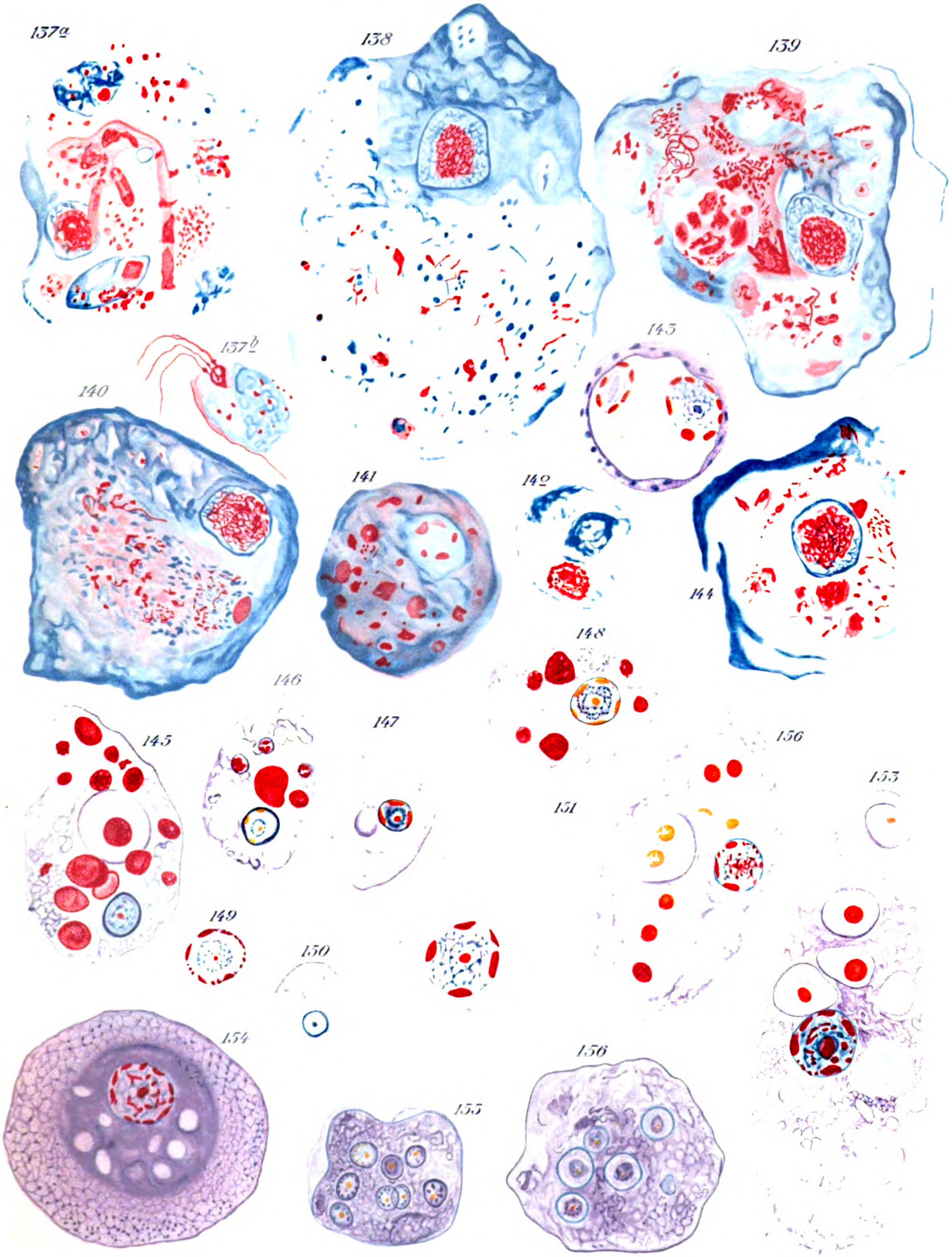
Figs. 151-153. Vegetative forms of *E. coli.* [Fig. 152 is inadvertently numbered 156 on the coloured plate. It is placed between Figs. 151 and 153.]

Fig. 154. Beginning encystment of a large vegetative *E. coli.* The size of the entamoeba shown here is unusual.

Fig. 155. *E. coli*, eight-nucleated cyst.

Fig. 156. *E. coli*, six-nucleated cyst.

PLATE XVII



Firenze Lit. A. Ruffoni, Piazza S. Croce 20

DYSENTERIES IN THE CANAL ZONE, WITH SPECIAL REFERENCE TO AMOEBIC DYSENTERY

BY

W. E. DEEKS, M.A., M.D.,
CHIEF OF MEDICAL CLINIC, ANCON HOSPITAL, ANCON, CANAL ZONE, PANAMA

(*Received for publication* 6 *January*, 1914)

CONTENTS

PAGE
Introduction 321
Amoebic Dysentery 324
  Aetiology of Dysentery due to infection with *Entomoeba histolytica* 325
  Pathology 328
  Symptomatology 330
  Treatment 333
  Summary 344
Dysenteries other than Amoebic 345
  Bacillary Dysentery 345
  Bilharzia Dysentery 347
  Balantidium Dysentery 347
  Malarial Dysentery 348
  Tubercular Dysentery 348
  Nephritic Dysentery 348
  Diphtheric Colitis 350
  Dysentery in the course of Pellagra 350
  Dysenteries resulting from the ingestion of decomposing meats, fish, etc. ... 351
  Dysenteries resulting from the ingestion of infected milks 351
  Dysenteries secondary to Cardiac or Hepatic Disease 351

INTRODUCTION

Dysentery means frequent bowel movements, attended generally by griping pains and tenesmus, and in the dejecta are to be found blood, pus and mucus.

These symptoms are the result of inflammation and ulceration of the rectum, sigmoid, colon, and of more or less of the small intestine. All or any part of these structures may be involved.

It is but a few years since all forms of dysentery were grouped together as acute and chronic; endemic and epidemic; or as tropical and otherwise, without any attempt being made to differentiate them on aetiological grounds; hence a variety of pathological conditions, due to as many different factors, were looked upon practically as one disease, and treated as such, solely for the reason that they had in common one symptom, namely, dysentery.

We now realize that there are many causes, some infective, and others toxic, and, unless careful differentiation is made, we are liable to err grievously in our management and treatment.

The term 'tropical dysentery' is also misleading, and has been regarded more or less as synonymous with amoebic dysentery. This latter affection is not limited to the tropics, but extends well into the temperate zones. It has been dreaded as much by the tropical resident as any of the endemic diseases there met with, and, directly or indirectly, has been responsible for a great many deaths.

We shall be able to show that this disease has lost its terrors, and plays a minor rôle in the forms of dysenteries met with in the Canal Zone, as far as its incurability, chronicity, and secondary effects are concerned.

The following forms of dysentery are more or less frequently met with in Ancon Hospital, and for the most part can be readily differentiated: —

(1) Amoebic dysentery caused by the *Entamoeba tetragena* of Viereck, or the *Entamoeba histolytica* of Schaudinn. These terms Dr. W. M. James, of this hospital, has proved represent one and the same organism. The former represents the quiet cyst-producing stage, the other, or *histolytica*, the acute vegetative stage. The consensus of opinion at present is that *E. histolytica* is the proper name for the pathogenic species.

(2) Bacillary dysentery caused by Shiga's or Flexner's bacillus, and the allied varieties.

(3) Bilharzia dysentery caused by the *Schistosoma mansoni.*

(4) Balantidium dysentery caused by the *Balantidium coli.*

(5) Malarial dysentery in the course of a general malarial infection.

(6) Tubercular dysentery due to tubercular ulceration of the intestine.
(7) Nephritic dysentery, or dysentery associated with acute diffuse nephritis, or secondary to a chronic nephritis with an acute process superadded.
(8) Diphtheric dysentery or colitis, associated with a diphtheric or gangrenous inflammation of the mucous membrane, of the whole colon, rectum, and part of the adjacent small intestine—a very fatal form.
(9) Dysentery in the course of pellagra.
(10) Dysentery resulting from the ingestion of decomposing meats or fish.
(11) Dysentery resulting from the ingestion of infected milk. These (10 and 11) may be bacillary in character.
(12) Dysentery, secondary to cardiac or hepatic disease.
(13) Dysentery associated with typhoid ulceration of the bowel.
(14) Clinical dysentery, which comprises the greatest number of cases and embraces that large group, of undetermined etiology, which occur here usually about May, in large numbers—about the close of the dry and the beginning of the rainy season. No microscopical or cultural findings have enabled us to determine their aetiological factor. They, however, are not very severe, lasting usually from four to ten days, and requiring no specific treatment. If, however, any of these dysenteries be associated with *Entamoeba coli*, to the uninitiated they are grouped as amoebic dysentery, and hence give rise to mistaken statistics.

A brief glance at the above clinical and pathological classification will suffice to show how important it is to ascertain the aetiological factor underlying the dysentery concerned before treatment is initiated.

One reason why there is such disparity of opinion between different authors as to the best methods of treatment is because the aetiological factor is not determined before treatment is begun, and they fail to appreciate the fact that dysentery is but a symptom, and not a pathological entity.

During the year 1911, one hundred and ten cases of all classes of dysentery were admitted to Ancon Hospital, and of this number only twenty-one were due to *Entamoeba histolytica.*

During 1912, two hundred and twenty-four cases were admitted, and of them forty-one were amoebic in character.

Owing, however, to the important part that this form of dysentery plays in the sick rate in the tropics, its recurrence, chronicity and complications, the greater part of this paper will be devoted to the method of treatment here adopted and the results obtained.

AMOEBIC DYSENTERY

Two distinct varieties of entamoebae have been recognized here as parasitic in the human alimentary canal, namely, the *Entamoeba tetragena* of Viereck, or *histolytica* of Schaudinn, and the *Entamoeba coli* of Schaudinn. Of these two, the last is apparently harmless, having been found in the dejecta of dysenteric and non-dysenteric patients alike. In the latter, we know that it has persisted for at least four years in two patients under constant observation, without having given rise to any dysenteric symptoms. It can, therefore, be dismissed as a pathological entity. During 1912 five cases of *Entamoeba coli* were met with, and these gave no pathological symptoms.

As to *Entamoeba histolytica* vel *tetragena.*

This is the form met with here most frequently, and is responsible for all our cases of amoebic dysentery. If not properly treated, it tends to relapse and to become chronic, and also by its metastatic development, is responsible for nearly all, if not all, of the hepatic, pulmonary and brain abscesses. It is this form of amoebic dysentery which has given us brilliant results with the bismuth subnitrate treatment, as will be shown later.

In 1911, Dr. S. T. Darling concluded that all of the amoebic dysentery here was due to *Entamoeba tetragena.* Since then, Dr. W. M. James, of this hospital, has done a great deal of work in endeavouring to clear up the situation. The following conclusions have now been definitely reached, and the question, I believe, to be definitely settled. Schaudinn based his differentiation of the species *histolytica* on its method of reproduction by means of budding. James has here shown that these so-called budding forms are merely degenerative changes which appear in the vegetative forms of *tetragena* when the stool is allowed to stand a couple of hours. He has specimens to show all the varieties of so-called reproductive budding which Schaudinn described. The *tetragena* of Viereck reproduces by four-nucleate cysts, and these can be obtained in every case when suitable conditions exist.

When *Entamoeba histolytica*, which may now be considered as the only pathogenic form of entamoeba, is in a suitable medium for development, and there is a corresponding severe dysentery in the patient, then the vegetative forms flourish to a degree, and division by fission ensues. If, however, the dysentery is not severe, and the ulceration is limited and semi-solid or solid stools result, then conditions are unsuitable for its reproductive growth by fission, and cyst formation takes place. In other words, it assumes a form suitable for its prepetuation and reproduction in another host, or in the same host, if suitable conditions recur.

The two clinical forms seen here represent merely mild infections with limited ulceration, or severe infection with extensive ulceration; in all cases the *tetragena* being the responsible pathogenic organism. In the milder cases, rest in bed and a milk diet is frequently sufficient to clear up the dysentery, and a condition of latency ensues, but repeated doses of Epsom salts will bring about a recurrence of the dysentery with a reappearance of the entamoebae in the stools unless the lesions are perfectly healed. It must be carefully noted that the disappearance of the dysentery does not necessarily mean a healing of the lesion, which may remain chronic for years, and be responsible for metastatic abscesses.

It is barely possible that there are two types of pathogenic entamoebae, but if such be the case, they both form four-nucleate cysts, and are difficult to differentiate morphologically, and impossible clinically.

AETIOLOGY OF DYSENTERY DUE TO INFECTION WITH *ENTAMOEBA HISTOLYTICA*

The organism is no respecter of persons. As seen here, all ages from eighteen months to sixty years, all nationalities, races, and both sexes, are prone to be affected in equal degree, dependent on the ingestion of the organism, or its cysts, under suitable conditions for its development. What these conditions are we know not, nor do we know definitely the source of infection. Three methods have been suggested; through fresh uncooked vegetables, through water, and third, by Darling, through direct infection, as typhoid might be transmitted by some carrier.

The last method is suggested by an infection which happened here to one of the laboratory assistants who was in the habit of examining the stools, and no other source of infection could be determined.

One would naturally think, however, that if this method of infection were common, the orderlies particularly, who handle the stools, and are in close contact with the patients, would be more frequently infected. We know, however, of no infection, either among them or the nurses in attendance on these patients.

As to infection through uncooked vegetables, the evidence I have to submit is of a very general character. We know that the disease was very common in Panama previous to the American occupation, and that now it is exceedingly rare. Green vegetables were then scarcely used because of this fear. Now, on the contrary, the use of green vegetables is very general, and the disease has practically disappeared.

Dr. Crabtree reports, however, the development of five cases in connection with the Culebra Penitentiary, traceable, he thinks, to the defecations in the yard, of a negro woman who had the disease. The men were in the habit of eating raw vegetables grown in this yard. It is quite possible that the cysts can live and be transmitted in this way.

As to the infection through water: Some object to this possibility because they have been unable to grow the organism in water media, or even keep it alive. This, however, does not preclude the possibility of cysts being harboured in water under conditions unknown to us, and developing in the human host when ingested. Because we are unable to do a thing, does not negative the possibility of its being done. We have evidence to show that water is a possible source of infection. Since the water supply has been installed in Panama, and the cisterns abolished, amoebic dysentery has almost disappeared. Prior to this the drinking water was taken from water tanks and wells in the yards. There was no sewerage system. The dejecta were either carried to the sea-wall and emptied at night, or thrown into the streets and back yards. One can readily see how soil contamination was thus favoured, and probably the wells also into which the surface water drained. This condition of things has now disappeared, and with it the dysentery. The only recent cases I have attended in Panama were those who have abandoned the use of the regular water supply for cistern water. Three of these cases were in bottle-fed infants taking only a sterile food and cistern water, and the other in an adult in the same house with two of these children. This evidence is most general in character, and inconclusive, but, taken in conjunction with the rapid diminution in numbers of the disease, coincident with the improved sanitary conditions, it certainly seems suggestive.

Our cases come now, for the most part from outlying districts where cisterns, springs and still river-water is used.

There appears to be no endemic centre. Every village on the Isthmus contributed one or more cases, generally, in proportion to the population.

Neither does length of residence play any rôle. Cases have been admitted here who have not lived over one month on the Isthmus, and others who lived here twenty or more years were admitted for the first time with this affection.

There appears to be no acquired immunity here against the disease.

The seasonal variation appears in the accompanying table of the patients admitted to Ancon Hospital up to the end of December, 1911. As can be seen, the prevalence is greater in March, April, and May, though it is always with us. In 1907, after the great influx of labourers, and before sanitary methods were perfected, we had the greatest number of admissions, 88. They were low in 1908, and increased again in 1909 and 1910, and lessened again in 1911, and increased in 1912. Two explanations exist for this: first, a better knowledge of the affection and a more patient search for the organism; and second, during the last three years the coloured labourers have been permitted to live outside of the Commission quarters, where the sanitary regulations and water supply cannot be so thoroughly supervised.

	1905	1906	1907	1908	1909	1910	1911	1912
Total Hospital Admissions	7,666	13,172	14,012	15,738	18,531	20,122	22,275	21,063
January	1	2	5	5	5	2	2	0
February	1	2	9	1	3	3	4	1
March	0	3	12	4	7	2	1	3
April	2	3	14	1	5	3	0	6
May	0	0	10	1	7	6	2	6
June	1	2	11	3	7	3	1	7
July	1	8	6	3	0	3	2	2
August	0	9	5	3	0	5	1	9
September	0	6	6	2	3	4	2	0
October	1	5	5	0	1	3	4	1
November	2	6	3	2	4	2	0	3
December	1	9	2	2	2	1	2	3
Totals	10	55	88	27	44	37	21	41
Deaths	3	20	26	5	3	4	0	6
Deaths, per cent. ...	30	36	29	18	6·8	10·8 (2·9)	0	14·6 (5·4)

In summing up the aetiological factors the only ones of importance appear to be *Entamoeba histolytica*, probably water borne; occupation which necessitates drinking the same; and possibly some condition of the intestinal tract or some symbiotic organism there, which aids in the elaboration of the nutritive material essential for the growth of the specific organism.

PATHOLOGY

We have found, in confirmation of the work of others, that the lesions are most common in the caecum and sigmoid, but in severe cases the whole colon, sigmoid, rectum, and even a small part of the ileum, adjacent to the caecum, may be involved, corresponding precisely to the distribution of the putrefactive bacteria.

According to Craig: 'The most characteristic lesions of the early stage of entamoebic infection are small nodular areas which project from the summit of the folds of the mucous membrane into the lumen of the intestine. The mucous membrane covering them is generally inflamed, and when incised these areas are found to contain a yellowish, or greenish-yellow, viscid fluid of a gelatinous consistency, which, upon microscopic examination seemed to be composed of a degenerated cellular material, mucus, and active motile amoebae. These nodular elevations mark the situation of the ulceration which develops later.'

These necrose, and, as Councilman and Lafleur and H. C. Clark, have shown, the resulting ulcers are at first coin-shaped, and about 1 cm. in diameter, involving the mucosa and sub-mucosa, and filled with a muco-purulent viscid material occasionally mixed with blood. The edges are undermined and hyperaemic.

In severe cases these small ulcers coalesce and form extensive sloughing processes extending to the muscular coat, which they occasionally penetrate. The serous coat may also be involved, thus causing perforation and general peritonitis.

The associated blood vessels are usually thrombosed, but mild, or grave and even fatal, haemorrhage may occur.

As stated above, the whole large bowel may be involved, which then appears as an oedematous, honeycombed, necrotic sloughing mass, in the tissues of which are the entamoebae and symbiotic, or associated organisms.

Kruse and Pasquale have isolated several varieties of colon bacilli found constantly present in the intestines of fatal cases, and have obtained cultures from the solid organs of staphylococci, streptococci, *Diplococcus pneumoniae*, colon bacilli, typhoid, and *Bacillus aerogenes capsulatus*. Some of these were undoubtedly terminal infections, and not directly concerned with the production of the amoebic lesions.

Dr. Clark has drawn attention to the frequency of the involvement of the appendix, in seven out of seventeen autopsies; and he says that repeatedly this has been the only location where the entamoebae have been found. This will be referred to later on in connection with the treatment.

In the dejecta of amoebic patients we find *Cercomonas* and other flagellates; but they are not constant, and are found when amoebae are not present. Associated also are frequently seen, particularly in the West Indian and native population, eggs from the *Trichocephalus*, *Ancylostoma*, *Strongylus*, *Ascaris*, and *Oxyuris* worms, and rarely from the *Bilharzia*. These are, however, incidental infections, and bear no relation to the presence or development of entamoebae, unless the mechanical lesions they may cause in the mucous membrane may form a point of entrance for the specific organisms.

In some autopsies the lesions are so extensive that they seem scarcely to have been compatible with life.

As is well known, the physiological functions of the large intestine are concerned with the final processes of digestion, absorption and elimination. Here takes place the final act in the drama of enzyme and organized fermentation, where the fermentative and putrefactive bacteria play an important rôle; also the liquid contents of the bowel are here absorbed, and the solid parts concentrated into mucus-covered faeces.

It can readily be appreciated that in extensive lesions the physiological function of the bowel is interfered with, and dysenteric stools result; while, on the other hand, when only one or more small entamoebic ulcers exist, the bowels can functionate, formed stools occur, and even constipation be a feature of the disease.

SYMPTOMATOLOGY

Latency has been a feature of some of our cases here, two of which were operated upon for chronic appendicitis when amoebic ulceration of the caecum in close relationship to the appendix was found. In these cases no history of dysentery was obtained other than that of an occasional watery stool. Dr. Herrick, in his report on hepatic abscess, states that over twenty per cent. of his cases have no history of previous dysentery. This certainly proves the latent character of the disease, and it may be present, and form small ulcerations insufficient to produce dysentery, and still be a grave menace to the individual because of its proneness, under special conditions, to produce metastatic abscesses in the liver.

The dysenteric forms of entamoebic dysentery are acute and chronic. In the acute type the symptoms are mild or fulminating in character. In the mild types the stools are from four to twelve in twenty-four hours. There is little prostration, practically no fever, and tenesmus only if there are lesions in the rectum. There is generally some anorexia, a coated tongue, and marked tenderness over the site of the lesion. As a rule these cases yield very readily to treatment. The milder forms pass gradually into those of the acute or fulminating type, where the prostration, distress, and number of stools daily may be choleric in character, and the tenesmus almost constant. In these cases the dejecta for the most part consist of necrotic mucous membrane, pus and blood-streaked mucus, in which the specific organisms and bacteria are numerous. The temperature in uncomplicated cases is never high, even in severe cases seldom reaching 102. It generally ranges from 98 to 100. It may be formulated that a temperature persisting over 100 is not characteristic of uncomplicated amoebic dysentery.

There is one other symptom almost invariably present in amoebic dysentery which is of considerable diagnostic and prognostic importance, and that is the doughy inelastic skin. When a fold is caught up and released, it crawls more or less slowly back into position. We have termed it here *myxenoid*. It is not symptomatic of amoebic ulceration alone, but is present in every ulcerated, necrotic, or chronic inflammatory condition of any of the abdominal viscera in greater or lesser degree. Its disappearance corresponds with the convalescence of the patient, and it is one of the best indications of his satisfactory progress.

Tenderness can always be elicited over the site of the ulcerated lesion, which most frequently affects the caecum, sigmoid, or rectum. If the lesions are very extensive, the tenderness is marked over the whole colon, and is in marked contrast with that over the small intestine. This forms a differentiating point between this and some forms of enterocolitis.

When the lesions are confined to the caecum, as is frequently the case, it is almost impossible to differentiate from sub-acute or chronic appendicitis, without a careful examination of the dejecta after the administration of a dose of magnesium sulphate, which always favours the elimination of entamoebae, and facilitates the examination of the stools. In these cases the leucocyte counts and temperature resemble closely those of the non-suppurative forms of appendicitis. The microscopical findings of entamoebae, blood, pus and mucus in the dejecta are diagnostic.

The course of these acute infections is variable. They may prove fatal in a few days with a sloughing, gangrenous, or perforated bowel, and subsequent secondary infection. In some of these cases before the fatal termination takes place showers of entamoebae are liberated from the lesions, and flood the liver, causing myriads of small necroses or focal abscesses.

In more favourable cases, the course is from one to four weeks after which convalescence rapidly ensues. Favourable progress is noted by the lessening in the number of stools, the cleaning tongue, the disappearance of toxic symptoms, and in the recovery of the elasticity of the skin.

In the chronic cases, which may persist for years, dysenteric symptoms are present with periods of remissions and exacerbations. There are digestive disturbances and some emaciation. The tongue is heavily coated, and the inelastic skin is present to a marked degree, also the localized tenderness. The patients are frequently anaemic and mentally depressed. In the chronic solitary ulcers there may be only vague pains and digestive disturbances with an occasional watery stool, but no dysentery. There is usually slight fever only.

Leucocytosis is variable, and the polynuclear leucocytes are increased. This is not characteristic, and depends on the amount of confined suppuration in the intestinal wall. For example, in one uncomplicated case, four leucocyte counts varied from 9,000 to 33,000; in another, in three counts, from 6,500 to 8,600; and in a third, in six counts, from 9,000 to 24,000. In an apparently uncomplicated case we had leucocyte counts varying from 3,200 to 39,000. Such variation precludes the possibility of leucocyte estimations being of any value in the differential diagnosis.

The acute diseases most frequently complicated with amoebic dysentery are malaria and tuberculosis. Pneumonia caused one death in our series of 323 cases; a complicating syphilis was responsible for another; and two died as the result of complicating pernicious malaria. Twenty-three per cent. of the deaths were due to complicating liver abscess—single or multiple.

Though the liver is the chief organ for the metastatic development of the organism, still we have had five pulmonary entamoebic abscesses and one cerebral. These, however, were secondary to liver abscesses. In one case operated on for pulmonary abscess, secondary to an hepatic abscess, the latter had completely healed, a prominent cicatrix marking its site.

TREATMENT

In regard to treatment. On November 13, 1909, Drs. Shaw and the writer made the following statement in the 'Medical Record,' published in New York:—

> A variety of methods have been tried in this hospital with varying success. They resolve themselves into oral medications and local treatment in the form of irrigations. Among the former may be mentioned castor oil, magnesium sulphate, opiates, ipecac, and bismuth and opium. Among the latter quinine irrigations in varying strengths, but usually 1 to 500, thymol irrigations 1 in 2,000 or 3,000, combinations of both, silver nitrate 1/4 per cent. boric acid, starch and laudanum, tannic acid, copper sulphate, normal salt, and plain water, warm and cold.
>
> Of all the different methods used, one stands out pre-eminently, that is, bismuth by the mouth and simple irrigations, either normal saline or plain water per rectum. The method is simple, harmless, and physiological, and, as our statistics show, more than justifiable. Absolute rest is enjoined, an absolute milk diet, of which there should be an abundance, saline or water irrigations, and bismuth sub-nitrate in heroic doses. We do not object, at the beginning, if tenesmus and distress be very severe, to an occasional hypodermic of morphine and atropine, but as a routine measure it is not considered good treatment.

This method of treatment was begun in 1908, and the uniformly satisfactory results were such that it remains to-day our standard method. Of the last 68 cases treated in this manner, extending over a period of thirty months, we have not lost an uncomplicated case. Further, of 190 cases treated by this method, we have had only three traceable relapses and one liver abscess.

Any method of treatment to be of value must be physiological. That the above meets this requirement we will now endeavour to prove. The responsible organism, that is, the *Entamoeba histolytica*, and the pathological condition have been described above, also the probable symbiotic character of the organism; that is, the associated growth of some putrefactive or fermentative bacteria. In this connection, it is interesting to note that the faecal odour in the bowels is confined to the colon, or extends but slightly into the ileum, and that the location of the amoebic ulceration corresponds precisely. It has been stated above that the lesions are generally confined to the caecum, sigmoid, and rectum. This can be explained on the basis that they are the more dependent parts of the bowel; or that it is in the caecum where the entamoebae first come in contact with the putrefactive bacteria, there meeting the organisms necessary to their existence; and it is in the rectum and sigmoid where the faeces with their bacterial content are longest in contact with the mucous membrane of the bowel.

It is with a consideration of these conditions, that we advocate, after a preliminary dosage of castor oil, the following:—

First: Rest, in order to increase the patient's resistance and give the minimum of movement to the bowel. This is classical treatment in all acute infections.

Second: A generous milk diet, because it is a physiological nutritious diet, admits of a minimum of intestinal putrefaction, and is practically all absorbed before it reaches the large bowel, which, owing to its ulcerative condition, is more or less physiologically inert.

Third: Saline or plain water irrigations one to three daily, purely for the purpose of lavage in order to rid the bowel of toxic products.

Fourth: The administration of bismuth subnitrate in heroic dosage. We give a heaped teaspoonful, equivalent to about 180 grains by weight, mechanically suspended in almost a tumbler of plain, or better effervescent water, every three hours, night and day in severe cases, only lessening the amount when improvement takes place. The mechanical suspension in a large amount of water is essential, otherwise it is prone to form a paste or solid mass, thus lessening its physiological effect. When the stools begin to lessen in number, and the tongue becomes clean, the number of doses is lessened to three or four daily. In very chronic cases it is wise to continue one or two doses daily for a month after convalescence is established. The absolute milk diet is not departed from until the tongue cleans, the tenderness over the bowels disappears, the elasticity of the skin returns to normal, and the stools have been reduced to one in 24-48 hours; then a normal diet may be gradually resumed as in convalescence from typhoid. We do not object to, but recommend, plain fruit juice once or twice a day instead of the milk during the acute attack.

How does bismuth subnitrate act? In 1883, Theodore Kocher demonstrated that the insoluble preparations of bismuth were actively antiseptic to fermentative and putrefactive bacteria. It is further known, clinically, that on the mucous membrane of the intestine, bismuth has a local sedative and astringent action. In our former paper we suggested that the curative power of bismuth subnitrate in amoebic dysentery rested largely on its antiseptic properties to the symbiotic putrefactive or fermentative bacteria. Now, however, we are led to believe that the process is more elusive than we had anticipated. Dr. S. T. Darling, in the laboratory here, has shown that the bismuth salt locks up the free sulphur on the surface of its crystals and even seizes upon the nuclear sulphur of the bacteria, turning them very black. It also affects the general flora of the intestines, destroying certain organisms and influencing others. Dr. W. M. James, of this hospital, has shown that the bismuth salts have a direct influence on the entamoebae, and also on the bowel contents (which I have frequently observed and directed attention to), not only destroying the characteristic odour, but liquefying the stool by ridding it of the mucus through liquefaction or inhibition of its production. The entamoebae show early degenerative changes. They become vacuolated, swell up, and the nucleus disappears.

After a few days, depending upon the severity of the lesions, the free sulphur resulting from bacterial decomposition of proteins is all combined, and the bismuth salt is seen in the dejecta scarcely changed in character. In two instances only in our experience have entamoebae been observed in the stools after four days' treatment by the bismuth method, no matter how grave the condition was when the treatment was begun. In one of these cases entamoebae were found alive on the fifth day, but not afterwards, and in the other, after disappearing on the third day, they reappeared again on the eighth day in large numbers, evidently, from the discharge of an entamoebic abscess from the intestinal wall, as the patient afterwards made an uninterrupted recovery, but returned two months later with a liver abscess as referred to later on.

Untoward effects of large doses of bismuth are frequently referred to in the literature. We have had five cases here showing a form of bismuth subnitrate poisoning. Two were in patients suffering with diffuse nephritis, and associated acute colitis; one in carcinomatous colitis, and one in a case of pernicious malaria with diarrhoea, and one in an amoebic case. The patients became cyanotic, but with full pulse and good heart action. Though the symptoms were alarming, they recovered at once after the administration of magnesium sulphate. The phenomenon was probably due to the liberation of nitrous oxide, or some similar gas, in the blood, through the decomposition of the bismuth salt.

One more drug should be referred to, because of its general use in the treatment of this affection; we refer to opium and its derivatives. Though an occasional hypodermic of morphine and atropine may be administered at the onset of the attack, particularly if the tenesmus is severe, still we deprecate its general use in the treatment of this affection because (*a*) it interferes with the production of enzymes and enzyme digestion, and (*b*) because it lessens intestinal peristalsis, thus favouring the retention of toxic products which irritate the normal mucous membrane, and which we should endeavour to eliminate. Exceptional cases are occasionally met with, in such extreme conditions of emaciation and exhaustion, and showing such toxic symptoms that it is not wise to temporize, but seek immediate surgical interference. The method which has given here the most satisfactory results, is that devised by Dr. A. B. Herrick, of this hospital. He performs a wide open caecostomy. It has three great advantages; (1) it prevents any ingested food from reaching the ulcerated surface of the colon; (2) it admits of a more generous and varied diet; (3) it enables free irrigation of the large bowel, so as to wash out toxic products. Its sole objection is the length of time it incapacitates the patients, because a reparative operation is necessary later. This, however, is of small importance, as by its means alone some lives can be saved.

No matter what methods of treatment are advocated, the burden of proof as to their respective values rests upon the results, temporary or permanent. Particularly is this true of amoebic dysentery with its well-known tendency to recur or relapse. It will be our object now to analyze the results of methods of treatment conducted in Ancon Hospital from the beginning of 1905 to the end of 1912, a period of eight years, and trace as far as possible the previous and subsequent histories of the patients for relapses and complications.

During this period we have admitted 323 cases with a total mortality by all methods of treatment of 67 cases, or 20·7. They were admitted as follows:—

Total Admissions to the Hospital, Cases of Amoebic Dysentery and Percentage of Deaths

Year	Total admissions	Amoebic dysentery	Deaths	Percentage of deaths
1905	7,666	10	3	30
1906	13,172	55	20	36
1907	14,012	88	26	29
1908	15,378	27	5	18
1909	18,531	44	3	6·8
1910	20,122	37 (34)	4 (1)	10·8 (2·9)
1911	22,275	21	0	0
1912	21,063	41	6 (2)	14·6 (5·4)

It can be seen at a glance that notwithstanding our increase in hospital admissions, there has been, on the whole, a decrease proportionately of amoebic cases, and a very marked decrease in the mortality in successive years (1912 excepted). It has been argued that the lowered rate of mortality is due to improved sanitation. We are willing to concede that sanitation has wrought miracles in lessening the number of cases, but in mitigating their severity we join issue. Some of the cases admitted here could not be more severe and be alive, in fact, some have died within twenty-four hours after their admission. From July 1, 1909, to December 31, 1912, a period of 42 months, 115 cases were admitted, complicated, and uncomplicated, of all grades of severity. Necessarily, in a hospital of this size, not all the cases came under one method of treatment. In this series of 115 cases, it was fortunately so, because it enabled us to compare the results of the different methods adopted. One hundred and one of the cases were treated with the method we recommend, that is described above, which we shall indicate as the bismuth-milk-saline method of treatment. Five of these cases not making satisfactory progress had caecostomy operations performed.

Of these 101 cases there were five deaths, one a United States citizen of German birth, admitted March 7th, 1910, with a double tertian infection, in addition to his amoebic dysentery. His temperature on admission was 102. His urine contained hyaline casts, renal epithelium, pus, and albumin; evidences of a severe nephritis. His stool, in addition to the entamoebae, pus, blood, etc., had ciliated monads. His temperature reached normal on the second day after his admission, remained so on the third and fourth, and he died on the fifth day with a temperature of 104. Unfortunately, there was no autopsy, but the clinical course of the disease was not that of a straight amoebic dysentery, and the complications of a severe diffuse nephritis, with a double tertian infection contributed to, if they were not responsible for, the fatal termination.

During 1912, the following six cases died, and are briefly reported. There are but two of these cases considered as treated cases. One had two days' treatment, and the other three.

Case I.—W. M., coloured Jamaican, age 29, was admitted June 10th with a pulse-rate of 120, respirations 32; treatment was begun on the 11th, but owing to unsatisfactory progress and his grave condition, he was transferred to the surgical side on the 13th for a caecostomy. He died on the 15th. The autopsy showed acute and chronic ulcerative colitis and proctitis. Before his transfer he was passing large masses of necrotic tissue.

Case II.—S. H., coloured Jamaican, age 47, was admitted February 12th. At first his dysentery was looked upon as due to nephritis. It was recognised as amoebic on the 15th, and treatment instituted. Owing, however, to his extreme condition he was transferred for a caecostomy operation on the 16th, but died on the 17th. He had taken the bismuth treatment for one day only. At autopsy, in addition to the usual ulcerative colitis, he had chronic nephritis and ulceration of the duodenum with perforation into the head of the pancreas.

Case III.—N. P., coloured Jamaican, age 34, was admitted July 24. His condition on admission was fairly satisfactory, and treatment was withheld until the evening of the 28th. His pulse then rose to 120, and treatment was begun. After three days he was transferred to the surgical ward for a caecostomy. He died August 5th, having taken bismuth for three days only. The usual ulcerative lesions were found at autopsy.

Case IV.—C. D., Panamanian female, age 49, was admitted about noon, August 9th, and died at 6 a.m., August 11th. She was not treated with bismuth. The autopsy showed acute and chronic extensive nephritis in addition to the ulcerative lesions in the bowel.

Case V.—V. F., Jamaican, age 10, was admitted November 29th in extremis, with pulse of 158. All medication was rejected by vomiting, and he lived about four days after his admission. He was untreated, and there was no autopsy.

Case VI.—F. D., coloured Barbadian, age 44, was admitted June 22nd, with a history of eleven days' illness. He was found to be suffering from advanced and chronic interstitial nephritis and a marked arterio-sclerosis in addition to his dysentery. Several examinations failed to reveal entamoebae, and his dysentery was considered to be due to his nephritis. He died on July 11th, and in addition to his clinical findings was found to have amoebic ulcerations as well. He was not treated with bismuth.

In reviewing these cases, and summing up the cause of death in each case, it can be seen that Case 4 lived only forty-one hours after admission, and was not treated with bismuth. Case 2 had one day of treatment, but as was seen at autopsy, an ulcer in the duodenum and perforations into the pancreas, played an important rôle in the cause of his death. Case 1 was treated for two days, but was passing necrotic tissue before a caecostomy was done. Moreover, he was greatly emaciated on admission. Case 3 was also much emaciated on admission, and had but 3,640,000 red cells with 71 per cent. of haemoglobin. Case 5 was untreated owing to his extreme condition, as was also Case 6. The cause of death in the last case could be attributed as much to his nephritis as to his entamoebae.

If we include all six deaths, then the mortality is 14·6 per cent. Excluding the three cases not treated, and the one treated for one day only, then the mortality is 5·4 per cent. But none of these cases could be considered as average cases of the disease, and the prognosis of all was practically hopeless before treatment was instituted.

The increase of the number of cases over 1911 is interesting, inasmuch as it shows that the improved sanitary conditions in general have not lessened the number of cases nor the severity of the symptoms during the past year.

One factor which may have had something to do with the increase is the water supply. The last dry season was particularly long, and there was a shortage of reservoir water. This had to be supplemented with water from the Chagres and other rivers, where the same sanitary supervision is not exercised as in the regular supply. This is strikingly verified by the great increase in the number of cases in those months at the close of the dry season, April and May, and the three following months, in the beginning of the wet season. Thirty of the total 41 admissions occurring during these five months.

Leaving these cases out of consideration, we had 100 per cent of recoveries. Of the remaining ten cases of this series one was treated with quinine irrigations (1 to 1,000) in September, 1909, and returned with multiple liver abscesses the following January. Another with quinine irrigations (1 to 2,000) and bismuth subgallate, grains (X) with opium by mouth for seventeen days. He was admitted September 23rd, and recurred November 6th, of the same year. He was then given the treatment we recommend and has not since returned. One case was not dieted, but took large doses of bismuth at irregular intervals. He relapsed, and was later admitted to Colon Hospital. Two other cases died on the day of admission, and received no treatment but stimulation. One died of general peritonitis from an unrecognized liver abscess on the under surface of the left lobe which ruptured; another of a pernicious aestivo-autumnal malaria within forty-eight hours of admission; one of multiple liver abscess complicated also by chronic nephritis, one week after admission. This case was operated upon, and one large abscess drained.

This leaves two to be accounted for. One, a Panamanian, died on the 11th day after his admission with a complicating disseminated tuberculosis, secondary to ulcerative pulmonary lesions. The cause of death in this case can scarcely be attributed to amoebic colitis. The last case to be considered in this series was a Martiniquan admitted May 3rd, 1910. He was treated for ten days by the bismuth-milk-saline method, but not improving, a caecostomy operation was performed, and he died the next day. The autopsy findings returned were: emaciation, suppurative conjunctivitis, chronic aortitis, chronic adhesive pleuritis, acute splenic tumour, cloudy swelling of the kidneys and liver, and acute and chronic colitis (chronic amoebic and acute healing process). The interesting feature about this case is that notwithstanding this man's emaciated condition on admission, and his lowered powers of resistance, the autopsy revealed, on the twelfth day after admission, with the bismuth-milk-saline method of treatment, acute and chronic ***healing*** ulcers from which '*smears failed to show amoebae.*'

If, then, we leave the two cases that died within twenty-four hours after their admission out of consideration, the case that died from tuberculosis (and this was a complicated case), those that died from liver abscess and pernicious malaria, and four of the 1912 cases unrecognized or admitted too late for treatment, we have had in the last three and a half years ninety-two cases with three deaths, or 3·2 per cent.

That amoebic ulcers do heal under this method of treatment we have ample evidence to prove. As Dr. Clark informs us in three of the cases autopsied, when the dysenteric process was complicated by other diseases, but were treated by this method, two of them having had caecostomy operations as well, active pink granulations were found, running out from all the islets and strands of the mucosa not destroyed, which in some cases quite filled the ulcer. In this connection we will report the case of M. C., Hospital No. 86158, coloured, Jamaican, admitted October 11, 1910, with typical amoebic dysentery, and a sub-normal temperature which did not rise above normal until the fifth day. He was treated with the bismuth-milk-saline method, with a disappearance of the dysentery on the 11th day. On the fifth day, however, his temperature began to increase and intermit, and he finally died on January 21, 1911, with a general disseminated tuberculosis and chronic nephritis. The interesting feature of this was that the autopsy revealed a great number of old cicatrices throughout the caecum and rectum of the healed amoebic type. Here was a case of curing the amoebic dysentery, in the presence of developing fatal tuberculosis and chronic nephritis, and it is not a solitary example of this process. These healed cicatrices are stellate, pigmented, somewhat depressed, and contain a lacelike collection of arterioles, according to Dr. Clark, and the healed mucosa seems to functionate.

It has been stated above that the appendix is frequently involved, and is the only place in which Dr. Clark has been able to find amoebae at the autopsies of cases who had taken bismuth.

This is interesting, because the appendix is almost inaccessible to the bowel contents which filter in from the caecum. The caecum, however, is more accessible to treatment, and heals before the appendix, where the putrefactive bacteria can subsist only as long as suitable pabulum is provided for them from the caecum. When this is rendered antiseptic, and cannot be further supplied, then the bacteria must die, followed by the amoebae.

In 15 of the 23 cases autopsied by him, liver abscesses, single or multiple, complicated the dysentery. The complication is a very serious one, particularly if the abscess be of the acute fulminating type. They generally run a rapidly fatal course.

We have demonstrated that amoebic ulcerations do heal by the bismuth-milk-saline method of treatment, even under the most adverse complicating conditions; now what about relapses or recurrences?

As to relapses: In order to ascertain as far as possible the frequency of relapses, that have occurred in Ancon Hospital, we have examined carefully the records of our 323 cases as to the number of times of their admissions here, and the nature of their illnesses. We have been able to trace the records of 87 cases only, admitted more than one time when they were treated for dysentery. Some have undoubtedly left the Isthmus, and some have been admitted to other hospitals. Of these 87 cases, with one or more subsequent visits to the hospitals, 66 were treated by the bismuth-milk-saline method as we recommend, and we find three recurrences following. In other words, 4·5 per cent. of relapses. Of these, one had been previously treated with quinine irrigations.

During 1912, we met with three cases which relapsed. One of these cases is the one referred to as Case 5. This patient was treated in March, 1911, in this hospital, for amoebic dysentery and a continuous fever, probably enteric in character. The child was also anaemic at that time, having 2,240,000 red cells and 39 per cent. of haemoglobin. This was only increased to 53 per cent. when his parents asked for his discharge, against the physician's advice.

The general fact holds good in this as in other chronic affections, that it is impossible to bring about an absolute cure if the general health of the patient is much below par. In this class of patients, where intestinal parasites are so common, and the nutrition is so low because of this and also because of insufficient nourishment, considerable time is necessary to bring about complete cures. In addition to this case, two Spaniards relapsed after treatment. One was a mixed infection, and a second bismuth treatment was given. Towards the end of his second visit to the hospital, the ipecac treatment was given, and since that time, though repeatedly under observation, no dysenteric symptoms have recurred, and his stools remain negative microscopically. The other case is an old man with chronic nephritis, and consequently low resistance. He apparently remained perfectly well after his second bismuth treatment.

Of those who had been treated by other methods in vogue here, such as irrigations of different kinds, bismuth subnitrate in small doses, with or without opiates, opiates alone, bismuth subgallate, etc., there were seventeen, of whom ten relapsed once or twice, or 59 per cent. This is eloquent testimony for the bismuth-milk-saline method of treatment. All of these cases had from one to eight subsequent admissions, and in almost all cases the stools were routinely examined at every admission. In those considered as cured, in subsequent admissions no history was given of having had dysenteric or diarrhoeal symptoms following their treatment.

What evidence have we as to the development of a liver abscess after treatment by the bismuth-milk-saline method? There have been 100 cases of liver abscess admitted to this hospital. In two cases only, this method of treatment was administered at a previous hospital admission, one for three days only. The other case was as follows:—

A. B., a coloured labourer, age 26, was admitted, March 7th, 1912, with amoebic dysentery. He was given the routine bismuth treatment, and no entamoebae could be found after the third day until the eighth day of treatment, when a great many were met with. He then apparently recovered, and was discharged on April 23rd. For two weeks previous to discharge he was running a little evening temperature, 99 to 99·6 at times.

On June 18 he was re-admitted with a liver abscess, benign in type from which, after evacuation, he made a good recovery. Probably the liver infection was present when he was discharged in April.

Of the remaining 19 cases admitted in 1912, twelve were admitted for the first time, and have no previous hospital records. The other six were admitted from once to six times previously, and although one of them had dysenteric symptoms six years previously, none were treated by the bismuth-milk-saline method.

We have every reason to feel, therefore, from clinical experience and pathological evidence, if the treatment be carried out as we have detailed above, that we can anticipate absolute cures, few relapses, and rarely a metastatic abscess. This holds good for acute as well as chronic cases. Our statistics show that those who sought hospital relief early, after the onset of the disease, were those most quickly cured, still chronicity is not a hopeless or deterrent factor, and relief quickly follows treatment. We have had chronic cases here, which were contracted in the United States, Brazil, and the Philippines. These had resisted treatment for years, but recovered promptly under the bismuth-milk-saline method of treatment. One case had been under treatment for eleven years, during which time he had formed stools for six days only. Twelve days' treatment in the hospital and about a month outside, with reasonable diet precautions, brought about a perfect result.

It has been stated above that *Entamoeba histolytica* is symbiotic, or associated with some other organism in its life history, and further, that metastatic abscesses are frequently found in the liver, and occasionally in the lungs and brain.

In the latter two situations, they are generally found secondary to those in the liver and are secondarily infected, thus having associated bacteria to elaborate their nutriment.

We need then consider only those abscesses which develop in the liver and which for the most part are sterile, showing no evidence of symbiosis, or associated organisms.

In explanation of this, it may be said that symbiosis means that the elaboration of metabolic products by the one is necessary to form suitable pabulum for the other organism.

The liver is the great metabolic gland of the body, where the absorbed products from the gastro-intestinal tract in the portal circulation are elaborated. It is more than probable that this medium is all that is necessary to form suitable nourishment for the entamoebae, when they have gained access to it through the portal circulation. It has been suggested that the entamoebae carry their symbiotic organisms with them when they form metastases. If this is true, then cultures ought not to be so frequently sterile. They are the result for the most part of necrotic liver tissue through the action of *Entamoeba histolytica*, which has on the tissues the action of an enzyme. We have seen two cases here where enormous sloughs slowly developed from an initial liver abscess. All the tissues melted before its progress, and no measures seemed to stay its course.

We believe that the frequency of liver abscess and its comparative absence in other organs, except when secondarily infected, can be best explained on this theory, that in the liver the cell metabolism elaborates a medium suitable for the development of the entamoebae which, in the intestines, require symbiotic or associated organisms.

We have, in conclusion, not only shown results, but have given a reasonable physiological explanation of them, and are willing to submit our results to other workers for criticism or approbation. No matter what may be said, the conclusions we have arrived at are drawn from, and rest on the analysis of case records.

SUMMARY

(*a*) Amoebic dysentery is due to a specific organism, the *Entamoeba histolytica* (*tetragena*), probably water-borne, and symbiotic in character.

(*b*) There is no endemic centre.

(*c*) Immunity, as we understand it, does not exist.

(*d*) Age, locality, nationality, or occupation, plays no rôle in the aetiology.

(*e*) The pathological lesions are peculiar and characteristic.

(*f*) Amoebicidal irrigations are useless.

(*g*) The bismuth-milk-saline method of treatment gives, in almost all cases, splendid results, if the lines indicated above for their administration are adhered to.

(*h*) Occasionally in extreme cases surgical interference, after Dr. A. B. Herrick's method, is indicated.

(*i*) This method of treatment gives a maximum of cures with a minimum of recurrences and metastatic developments, the most frequent of which is liver abscess. This offers a serious complication in the treatment, particularly if of the acute or fulminating type.

DYSENTERIES OTHER THAN AMOEBIC

In discussing dysenteries other than amoebic in the Canal Zone, it must be noted that difficulties in connection with the diagnosis frequently arise, and one is unable always to determine the exact aetiological factor. The following forms of dysentery have been shown to exist here, and will be briefly discussed, both from a clinical and pathological standpoint. Also, as far as we have been able to determine, the best method of treating them respectively.

BACILLARY DYSENTERY

Fortunately this form of dysentery is comparatively infrequent in the Canal Zone, but is of grave importance when met with. The organisms responsible for the affection belong to different strains of the colon group, and from our experience here are not readily recovered from the stools. Only four positive cases have been reported from our laboratory. In the 'American Journal of the Medical Sciences' of January, 1912, three of these cases were reported by Drs. Darling and Bates. There were two adults, and one child. Another infant, seven months old, was admitted December 15, 1911, from which the Y-strain was cultured from the stools. This patient recovered.

One of the Darling and Bates' cases was interesting in that the organism was recovered from a blood culture four days before death. The other two cases recovered. Clinically, I do not believe that this represents nearly all the cases of this affection which we have had in this hospital. Unfortunately, routine cultures from the stools have not always been made, and even then the recovery of the organism is very difficult. Patients suffering from this form of dysentery are usually more prostrate than the ordinary amoebic cases. They have more fever, often reaching 103 or 104 degrees, and have more blood in the stools. The bowel is generally infiltrated, and, in consequence, is much more readily palpable than in amoebic infection, and also more painful when palpated. They frequently run a febrile course suggestive of typhoid.

We have no specific for this form of dysentery. At present, we are getting good results from the use of opiates after a preliminary dose of calomel and salts, or castor oil. Opiates apparently act by lessening peristalsis, thus preventing the organisms from being thrown deeper in the tissues through the lymphatic system. The course of the disease varies from four or five days to two weeks, and recovery generally takes place.

During the year 1911 we had two deaths from ten admissions which were clinically of this class. Some of them, however, may been due to other causes. During 1912 there were clinically seven cases with two deaths.

The autopsy on one showed shallow, coin-shaped ulcers in the colon, appendix and small intestines, also in the sigmoid where one had almost perforated; acute and chronic vegetative, aortic and mitral endocarditis, acute degenerative nephritis, chronic myocarditis, infarctions in the spleen and acute and chronic lymphadenitis of the mesenteric, mesocolic, inguinal and femoral glands. This, with infarction of the spleen, shows that the dysentery is but part of a general systemic infection and septicaemic in character.

In the other there was acute diffuse colitis, enteritis and appendicitis, with exudation and oedema; lymphadenitis similar to the other case; cloudy swelling of heart, liver and kidneys; with meningeal congestion and haemorrhagic effusion into the stomach and intestine.

The diagnosis can always be suspected in the absence of a positive culture of the stool, by the presence of severe persistent bloody dysentery, accompanied by fever and a thickened tender bowel, when palpated.

BILHARZIA DYSENTERY

During the year 1911 three cases of bilharzia infection were admitted to Ancon Hospital, and during 1912 eight cases. One of these had an associated infection of *Entamoeba histolytica*, which probably played a greater rôle than bilharzia in the production of the dysentery.

Of the 1912 cases which showed bilharzia infection, six had dysenteric symptoms with blood and pus in the stools. Of the six, one died after 5½ months in the hospital with continuous dysentery, which no remedy appeared to relieve. Dr. S. T. Darling has reported the pathological findings of this case before the Canal Zone Medical Association. The colon was ulcerated and thickened, but this condition was tremendous in the lower end of the sigmoid and rectum, where it was riddled with suppurative sinuses, one of which had perforated and set up general peritonitis which was responsible for the lethal termination.

Dr. Walter Baetz, of this hospital, reported in the 'New York Medical Journal' of July, 1911, another of these cases. The dysentery may be very severe and very difficult of diagnosis, as was evident in this case. Only careful and continuous stool examinations will enable one to determine its true character. No specific remedies are known, all our cases here have recovered with the one exception.

BALANTIDIUM DYSENTERY

In recent years some attention has been given to *Balantidium coli* as a cause of dysentery. Recently Dr. Fred B. Boland, in the 'Journal of the American Medical Association' has written on its pathogenesis.

In Ancon Hospital during the routine examination of stools, it is met with from ten to fifteen times a year. It may or may not be associated with dysenteric symptoms. We have never attributed to it pathogenic properties, and have never had a fatal case in which it has been found. We generally meet with it here in mild forms of colitis, with associated flagellates and intestinal vermes, but have never thought that it played an important rôle in the production of the colitis. It is, however, a factor which must be considered when the aetiology of the tropical dysentery is under discussion.

MALARIAL DYSENTERY

In the course of an acute malarial attack it is not uncommon to find a fairly severe dysentery. Malarial organisms tend to localize in different organs, and not infrequently haemorrhages are found at post-mortem examinations into the mucous membranes of the stomach and intestines. Whether, however, the dysenteries are due to this or to malarial toxins has not been determined.

During the year 1911 we have had eight of these cases, all of whom recovered, and during 1912 sixteen. No special treatment is necessary. The ordinary routine malarial treatment suffices to bring about speedy convalescence.

TUBERCULAR DYSENTERY

This may occur in the course of a general tubercular infection, or the ulceration may be limited to some part of the intestinal tract. The diagnosis can only be made on the persistence of the dysentery, and on the presence of acid-fast bacilli in the stools, or more positive still, by the development of tubercular lesions in guinea-pigs after the injection of some of the specific organisms. There is nothing to add concerning the treatment of this class of cases. General hygienic conditions, such as are recommended for pulmonary tuberculosis, probably give the best results.

NEPHRITIC DYSENTERY

In Ancon Hospital, this comprises clinically a large and important group, and is about as frequent and more fatal than entamoebic infection. The aetiology is as yet not sufficiently clear to make any definite statement. It is impossible to state whether the ulcerated colitis is secondary to the kidney acute, and chronic degenerative changes, or whether they are both the result of some undetermined infection. Suffice it to say that it is an important clinical entity, and its successful management necessitates a thorough understanding of the close relationship.

The cases associated with the acute diffuse degenerative nephritis are usually very asthenic, toxic in appearance, running normal or subnormal temperatures. The pulse is exceedingly soft and of low tension, the sphygmometer generally registering about 100. The abdomen is retracted, the bowel not perceptibly thickened, and the skin without elasticity. The stools are numerous, watery and mixed with pus and blood, and there is a tendency to chronicity.

Generally the patients recover without leaving great damage to the kidneys. Occasionally, however, they drift into a chronic course, and the kidney lesions ultimately simulate in the clinical course the character of the interstitial type of nephritis. We have seen a patient of this character in the course of three months in a ward pass from one stage to another. The first few days he passed from ten to fifteen ounces of urine daily, and at the end of three months over 100 ounces.

During the past year we have had twenty-five admissions, with five deaths from this type of dysentery; two associated with acute nephritis, and three with chronic. The chronic nephritic cases had the acute process engrafted on them.

The aetiology is obscure. Some may follow a malarial infection. Every severe malaria has some nephritis, or, at least, cloudy swelling of the kidneys. It frequently happens that this nephritis is very severe, and may be followed by dysentery.

Another important factor in the production of nephritis is the diet. I believe that this is the greatest of all aetiological factors here. The coloured people, not living in Commission quarters, usually provide their own food. This consists, chiefly, of rice, yams, sweet condensed milk, and about once or twice a week, codfish or a little pork is added. This sweet, starchy food, deficient in protein, green vegetables and fruits, is conducive to intestinal fermentation, and resulting toxic products, which produce the nephritis and subsequent or coincident dysentery.

In this form of dysentery the ulcerative lesions are not confined to the caecum and large bowel, as in the amoebic and diphtheritic types, but the small intestine also is frequently involved. Neither are the lesions as destructive and characteristic as the abovementioned form.

The treatment differs radically also from the afore-mentioned types. It is in these patients that bismuth, if administered as recommended for amoebic dysentery, is prone to break up, forming nitrous oxide compounds, and symptoms of asphyxiation result. On the other hand, elimination must be favoured. Magnesium sulphate frequently administered has given the best results. It is frequently necessary also to give stimulation. For this purpose here we prefer nux vomica. The diet should be milk and fresh fruit juices during the acute stage, but later on proteins and green vegetables can be judiciously added.

As stated above, the prognosis is fairly grave because of the damaged kidneys.

DIPHTHERIC COLITIS

This is an interesting and very grave form of dysentery. During the year 1911 we had three deaths from this cause, and one in 1912. The lesions affect precisely the same part of the alimentary tract as entamoebic infection, but are very different in character. A diphtheritic gray-looking slough covers practically the whole of the mucous membrane of the large and a small part of the adjacent small intestine. Occasionally we have seen this complicating the lesions of entamoebic ulcerations, apparently as a terminal infection. No cultural findings here have been able to determine what the underlying factor is which causes this necrotic, diphtheritic condition. As far as we know it is always fatal. The symptoms of the patients show extreme prostration, usually considerable fever, very frequent bowel movements, consisting chiefly of blood. The haemorrhages sometimes are as great as one meets with in typhoid ulceration of the bowel; but in this case gushes from him at frequent intervals. The course of the dysentery usually is but a few days, and no remedy other than palliative is known.

DYSENTERY IN THE COURSE OF PELLAGRA

This form of dysentery resembles the clinical forms of dysentery frequently met with here. During the year 1912, seventeen cases of pellagra were admitted to this hospital. With very few exceptions all suffered from a more or less severe dysentery. Of the five fatal cases, only three showed ulceration of the bowel at post-mortem examination, and in none of them was the ulceration very severe. We have found here that a carbohydrate-free diet with dilute nitric acid before meals usually brings about a speedy convalescence. In two or three instances where the dysentery was very severe, teaspoonful doses of bismuth three or four times daily was found to be very useful.

DYSENTERIES RESULTING FROM THE INGESTION OF DECOMPOSING MEATS, FISH, ETC.

When meat, fish, shell-fish, crustaceans, and such highly nitrogenous food as beans, are partially decomposed, they are prone to give rise, when ingested, to a more or less severe colitis or enterocolitis due to bacterial infection or the resulting toxin. These bacteria are for the most part *Proteus vulgaris*, *B. coli*, *B. enteritidis*, and allied organisms. This group of dysenteries is much more important in point of frequency than is generally considered. They are frequently serious and occasionally fatal. In tropical countries, where there is much humidity, the difficulties in preventing meat decomposition are very great, particularly among the poorer people where ice is a luxury. The partial decomposition which these undergo, due to the bacterial infection, when ingested set up a train of symptoms similar to those of any severe dysentery. Frequently, high temperatures, griping pains, numerous stools with tenesmus, and complete anorexia are present. My opinion is that in the tropics more dysenteries are caused by contaminated food products than by any other single cause. Generally after a purgative, the condition slowly subsides, but the dysentery may last for four or five days.

DYSENTERIES RESULTING FROM THE INGESTION OF INFECTED MILKS

In June, 1909, thirteen people were poisoned here in Panama by eating ice cream. Ten of these were admitted to Ancon Hospital with temperatures ranging from 102 to 104, which persisted from two to four days. There was complete anorexia, in some of them, vomiting with much prostration. All of them had dysentery, which lasted from three to five days. I have met with other cases of dysentery here, due to the ingestion of infected milk, and have found that the symptoms above mentioned, particularly high temperature, anorexia and prostration, were generally present, and very characteristic. No specific form of treatment is indicated.

DYSENTERIES SECONDARY TO CARDIAC OR HEPATIC DISEASE

This form of dysentery is met with here, as elsewhere, and requires only to be mentioned.

From the foregoing conditions, it can be concluded that the subject of dysenteries is a large one, and much yet remains to be learned both as to etiology and treatment. In Ancon Hospital more attention has been directed to the amoebic type than the others, and we feel now, as far as *Entamoeba histolytica* (*tetragena*) is concerned, that its pathology is characteristic, and its treatment established. The exact mode of action of the treatment is still a subject for discussion, but the results are practically perfect.

The frequent occurrence of *Entamoeba coli*, which is harmless, undoubtedly is responsible for the discrepancy in results which have followed this or any method of treatment of amoebic dysentery. Many a patient has been submitted to disagreeable, tedious, and oft-times painful methods of treatment because of the failure first to diagnose the type of dysentery.

It must further be realized that dysentery is only a symptom, and not a pathological entity, and no single method of treatment is applicable to all varieties.

The great majority of the dysenteries met with here will improve, after the administration of a purgative, on rest in bed and milk diet, no specific being necessary.

It is to be sincerely hoped that in the near future we shall have some more definite scientific data on which to base our classification than clinical observations, but the practitioner in tropical medicine has no other alternative at present.

EMETINE IN AMOEBIC DYSENTERY

BY

W. E. DEEKS, M.A., M.D.

CHIEF OF MEDICAL CLINIC, ANCON HOSPITAL, ANCON, CANAL ZONE, PANAMA

(*Received for publication* 6 *January,* 1914)

Up to the time that Rogers published his papers on the use of emetine in the treatment of amoebic dysentery, we were using bismuth subnitrate very successfully in the treatment of this affection.

From all reports on the subject, I believe that the results obtained from it were incomparably superior to those by any other method, not only from the standpoint of immediate results, but also on the prevention of relapses and metastatic abscesses. Some of my results from its use were published in the Journal of the American Medical Association, January 4th, 1913.

Rogers' brilliant results with emetine induced me to try it here, and I wish now to report the cases so far treated.

CASE I. W. de C., aged 29, was admitted on May 13th with a history of two months' illness with dysentery. He was passing from ten to twelve stools daily. On examination he was found to have a severe case with the temperature varying from 99°F. in the morning to 101° or 102°F. at night. Dr. W. M. James determined the organism to be *Entamoeba histolytica* or *tetragena*, of which there were great numbers. The abdominal tenderness was marked; the skin inelastic; and there was a pleuritic friction rub over the right lung which probably accounted for the high temperature, which is unusual in uncomplicated amoebic dysentery.

On the 15th, two days after admission, he was given two 1/3 grains hypodermic injections of emetine. On the 16th, 5/6 of a grain in two injections; on the 17th, 1 grain in two injections; and on the 18th, one injection of 1/2 grain—3 grains in all. The temperature reached normal on the 18th and remained so.

Eleven stools were recorded on the 14th; thirteen on the 15th; six on the 16th; four on the 17th; and one daily subsequently. The amoebae disappeared from the stool on the 18th and were not subsequently found. The stools became normal on the 22nd, and the patient was discharged on the 24th apparently perfectly well, even the skin having reached its normal tonic.

CASE II. R. P., aged 27, native of Panama, was admitted June 20th with a chronic amoebic dysentery of eight months duration, during which time he had frequent exacerbations.

On the 22nd 1/3 of a grain of emetine was administered hypodermically; on the 23rd, 2/3 of a grain in two doses; on the 24th, 1 grain in three doses; on the 25th, 1 grain in three doses; on the 26th, 2 grains in two doses; and the 27th, 2 grains in two doses—in all, 7 grains. The amoebae disappeared on the 28th, and the pus from the stool on July 4th. He was discharged apparently well on July 7th.

CASE III. J. J. M., aged 27, white, was admitted June 24th, with a history of dysentery of eight months' duration, not very severe except during frequent exacerbations. He also had malarial parasites in his blood. On June 30th the emetine treatment was begun with two 1/3 grain hypodermics; on July 1st, 1 grain in three doses; and the same was repeated on July 2nd and 3rd, and again on July 6th. No amoebae were found after the first injection, and the stools were normal on July 2nd. In all he received 4-2/3 grains.

CASE IV. J. G., Colombian, aged 38, was admitted to the hospital on July 10th with a history of dysentery for two weeks, but previous to this was passing some liquid stools for another fortnight. On examination he was found to have an acute fulminating attack of amoebic dysentery. Treatment was begun on the 11th with 1/3 of a grain hypodermically; on the 12th, 1 grain was given in two doses; and on the 13th, 2 grains in two doses—in all 3-1/3 grains. Improvement from the first was rapid. The amoebae disappeared on the 14th, and the pus on the 18th.

I wish to refer to another case in which the action of emetine probably saved the patient's life.

This patient had been operated on for abscess of the liver. The abscess was very large, and probably of several weeks' standing. He was anaemic, weak, and had lost a great deal of weight. After the abscess was drained, the improvement was temporary only. The liver continued to slough, and large quantities of pus were daily evacuated. In addition, the cytolytic action on the tissues surrounding the sinus had begun, and the temperature rose to about 102° F. every night. This continued for nineteen days after the operation, and the condition of the patient was almost hopeless. He was operated on May 31st. On June 19th, 1/3 of a grain of emetine was administered; on the 20th, the same; on June 21st, 1 grain; and on June 23rd, 1 grain. The temperature became normal on the 23rd, and remained so for three days. On the 26th, the temperature again rose, but not as high as formerly. There was a daily rise to 101° or 101·5° F. until July 9th, when 1/2 grain of emetine was administered; on July 10th, 1 grain, and July 11th, 1-1/2 grains. The temperature then became normal, and has remained so. The patient is still (July 21st) in Dr. Herrick's clinic, is doing well, and has every prospect of ultimate recovery.

Another case, similar in character, but not so severe, in Dr. Herrick's clinic, had also an accompanying dysentery. Under the use of emetine he made a good recovery. I know of no other drug that could have brought about such good results.

These cases more than justify a continuance of the use of emetine, and the convenient way of administering it also is very advantageous. Nausea has been observed on only a couple of occasions, and this to no great extent.

How does emetine act? I believe it to be a direct poison to the amoebae, as quinine is to the malarial parasite. On no other ground can I explain the brilliant results with the small dosage given. In the majority of cases, I believe three grains to be sufficient, but in the more severe cases probably six to eight are required.

In one of the above-mentioned cases, though the amoebae disappeared under the use of emetine, there were four or five evacuations daily. The administration of bismuth in teaspoonful doses three times daily promptly brought about a perfect result. We now have records of twelve cases treated by emetine alone, and of these, we have had six relapses. The cases were given from six to twelve grains each, and relapses occurred in the larger as well as the smaller doses. There is no doubt as to the efficiency of the drug in relieving the symptoms, but our opinion is, from the cases cited, that it is better to combine bismuth with the emetine.

I believe that in the two drugs, either singly or combined, almost every case of amoebic dysentery can be cured, unless in terminal stages, when he or she submits to treatment.

While emetine probably acts as a direct poison to the amoebae, bismuth probably acts by destroying the symbiotic organisms necessary for their growth in the intestinal canal.

SIX DAY FEVER IN PANAMA

BY

W. E. DEEKS, M.A., M.D.

CHIEF OF MEDICAL CLINIC, ANCON HOSPITAL, ANCON, CANAL ZONE

(*Received for publication* 6 *January*, 1914)

SEVEN CHARTS

In the Journal of the American Medical Association of October 26th, 1912, the writer published 'A Preliminary Report of a Hitherto Unrecognized Six-Day Fever, in Ancon, Canal Zone.'

Since writing that report cases have accumulated, and up to the present time, fifty have been admitted. These have furnished sufficient data to enable us to give a more accurate account of this interesting fever, which is unique in the Western Hemisphere.

In the former report, it was stated that the epidemic first appeared in two mail clerks who exclusively handle the foreign mails. They sickened within an hour of each other, and no other cases appeared for ten days, which would indicate the incubation period of the disease. Since that time the affection has spread through the American colony in the vicinity of Panama, and also among the Panamanians, but has not been recognized among the coloured population.

In all, fifty cases have been treated in the hospital, and probably many more were treated outside. The mortality is nil, although some of the patients afflicted have been very ill.

As there have been no deaths, we are ignorant of the pathological lesions.

The disease appears to spread from certain centres. When one person in a household sickens, usually several in the same household or from the same vicinity soon acquire the disease, but not all. This suggests that either some are naturally immune, or that there is an infecting agent which does not convey the disease to all. Any sporadic case can always be traced to his or her having visited an infected focus, or possibly stayed over night, as was shown in the large bachelor quarters where the infection remained some time.

Several sporadic cases in neighbouring towns occurred, but these had in all cases, as far as could be determined, visited an infected focus.

Whatever the nature of the infection, it is interesting to note that none of the attendants on the patients in the hospital ever contracted the disease, though no special precautions were taken to guard against such infection. This argues that either the disease can be communicated in the pre-febrile stage only, or else if there be an infecting agent, this was not present in the hospital.

SYMPTOMATOLOGY

There are no prodromata; patients apparently well are suddenly seized, frequently with chills, and the temperature promptly rises to from 100° to 103° F., depending upon the severity of the disease.

Giddiness and vertigo are occasionally complained of, and intense frontal headache about the eyeballs is very persistent, as are also dull aches in the back, loins and legs. Occasionally these are severe enough to necessitate alleviation by medication. There are no joint pains.

Anorexia is constant and continuous throughout the febrile stage, and very frequently vomiting during the first two or three days.

PHYSICAL SIGNS

The tongue is usually coated, but not markedly, there is no sore throat. The eyes and face are usually injected, and sometimes markedly so.

The pulse is normal, or for the degree of fever, slow. Respirations are not altered. The spleen is usually enlarged and generally palpable, its free border projecting two or three fingers breadth below the costal margin.

The liver has not been found to be enlarged, as is so common in malaria.

The skin is frequently mottled or erythematous soon after the onset, but there is no characteristic or constant skin rash early in the disease.

The urine has frequently a faint trace of albumin, and a few hyaline and granular casts like most febrile albuminuriae. Sometimes, however, the nephritis is very severe, as was seen in three of our cases. The nephritis was of the diffuse type, with a great deal of albumin, hyaline, granular and epithelial casts, but never generalized oedema as in the nephritis of scarlet fever. In all cases the urine became practically normal before the patients were discharged. In severe cases the albumin disappeared within a week after the fever subsided, and only a few hyaline casts remained at the end of the second week.

The blood findings are interesting. In my former report there seemed to be some variance as to the leucocyte counts. This was due to the fact that some were taken early, and others late, in the course of the disease.

The following counts were taken within the first three days of the onset, and show how constant this feature of the disease is, and it has, in consequence, considerable diagnostic importance: —

LEUCOCYTES	POLYNUCLEAR	LARGE MONONUCLEAR	SMALL
5600	91	7	2
7600	80	18	2
5000	89	6	4
4800	91	5	4
3300	72	22	6
—	88	5	7
7800	84	6	10
5400	83	8	9
Leucopenia	78	10	12
7200	80	5	15
3500	80	3	17
—	90	5	7
6000	90	8	2
6360	88	8	4

Unfortunately, a number of other leucocyte counts were taken so late in the course of the disease that they are very variable, and of no value.

The above, however, gives one a good idea of the blood picture, as it shows, generally, a leucopenia with a high polynuclear differentiation.

The febrile stage of the disease varies from five and one-half to six days, and is very constant.

When the date of the onset is known, the defervescence of the fever can always be predicted within a few hours.

The temperature curve is of the characteristic saddle-back variety, as has been described by Rogers and Crombi, and also by Castellani. The intervening period between the onset and the fall of temperature is lower than at the beginning and the end of the febrile stage.

The accompanying charts show these characteristics (see pp. 362-365). The temperature usually falls by slow crisis; generally falling in twenty-four hours between the highest point of the febrile stage to normal. Sometimes this is accomplished in twelve hours.

In one instance only have we seen the fever last over six days. On the sixth day there was a pseudo-crisis, as in pneumonia, and the temperature reached normal and remained so on the seventh. In two instances, after the crisis a slight post-febrile temperature of from one to one and a half degrees persisted for three days longer.

THE RASH

This is almost always present, and is very characteristic. It usually makes its appearance on the dorsal surfaces of the feet, and the radial surfaces of the forearms, either the first or second day after the temperature reaches normal. The appearance of this rash may be delayed to the sixth day after the drop in temperature. It is finely petechial in character, and may be spread so as to involve all of the extremities. Rarely is it seen on the body.

In a recent case the petechial rash covered completely the body surfaces. Over the shoulder blades and buttocks, where there was closer contact with the bed, it was so thick as to give a vivid red colour to those parts.

The rash does not disappear on pressure, but when it begins to fade, gives to the skin involved a general dark bluish or cyanotic tinge. I have never seen any rash that resembled it.

In the cases above referred to, though the rash was so general and so severe, no desquamation followed.

It frequently is accompanied by a general erythema, which may involve the whole body. This blotchy erythema is intensely itchy, and frequently necessitates narcotics, in order to obtain rest. The rash remains out from one to three days, when it gradually fades. When the petechial form of the rash is very marked, it occasionally is followed by desquamation.

The petechial form of the rash is practically constant, but sometimes so slight that it may be overlooked, unless carefully sought for on the first and second days following the fall of the temperature.

In order to determine the nature of the infection, cultures of the blood, urine and stools were repeatedly taken by Drs. Samuel T. Darling, H. C. Clark, and Lewis B. Bates at different stages of the disease, and all proved negative.

The Widal reactions have all been returned negative.

Efforts were also made to transmit the disease to animals, without result.

The disease undoubtedly is a distinct entity, probably related to the exanthemata. If there be a transmitting agent, it has not been found here. It certainly is not a mosquito, as a great many cases have occurred here in mosquito-proof houses.

The infective agent is probably transmitted over long distances, as we have every reason to think that the first two cases derived their infection from sorting mails from India destined to Hindoo labourers here.

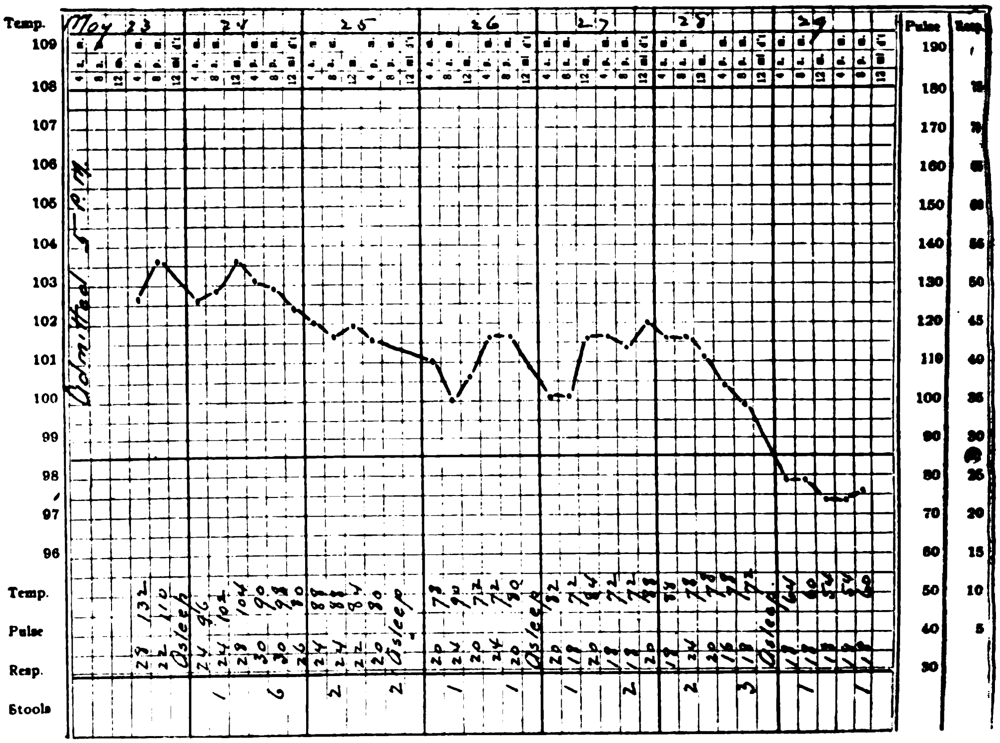
The disease is probably identical with the seven-day fever referred to by Castellani in his 'Manual of Tropical Medicine.' It has been described by Rogers and Crombi as occurring in the seaport towns of India and Ceylon during the months May to September.

Leonard Rogers describes cases with a duration of seven and more days, and says that blood cultures in six of his cases were positive for a bacillus related to the *B. coli* group. As Dr. T. R. Brown and I have shown here, it is not unusual to have paratyphoid with seven and eight days of fever. I am inclined to believe that Rogers has included some of these in his description of seven-day fever.

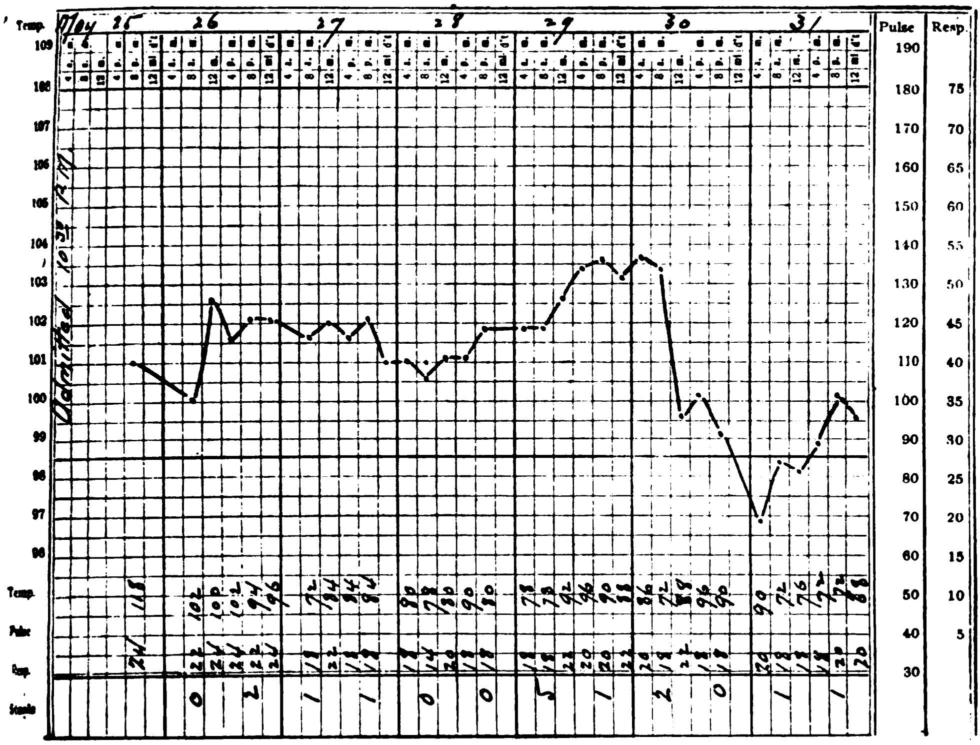
The disease can readily be differentiated from malaria by the absence of parasites in the blood, and the differential leucocyte count, also by its failure to yield to quinine; from dengue by the absence of joint pains, by its being a single phase fever, and by its large spleen, which is never present in dengue, and the characteristic rash, also the time it occurs in relation to the fever; from influenza, by the absence of coryza and pulmonary symptoms, the blood picture and the definite course; from typhus, by the febrile course, rash and milder manifestations; from typhoid and the paratyphoid group by the cultural blood findings.

In conclusion, it may be stated that we have at present a distinct disease, running a characteristic febrile course accompanied by a rash, probably allied to the exanthemata, which made its appearance in Ancon Post Office last April, and which is still with us. It has not been recognized hitherto or described in the Western hemisphere.

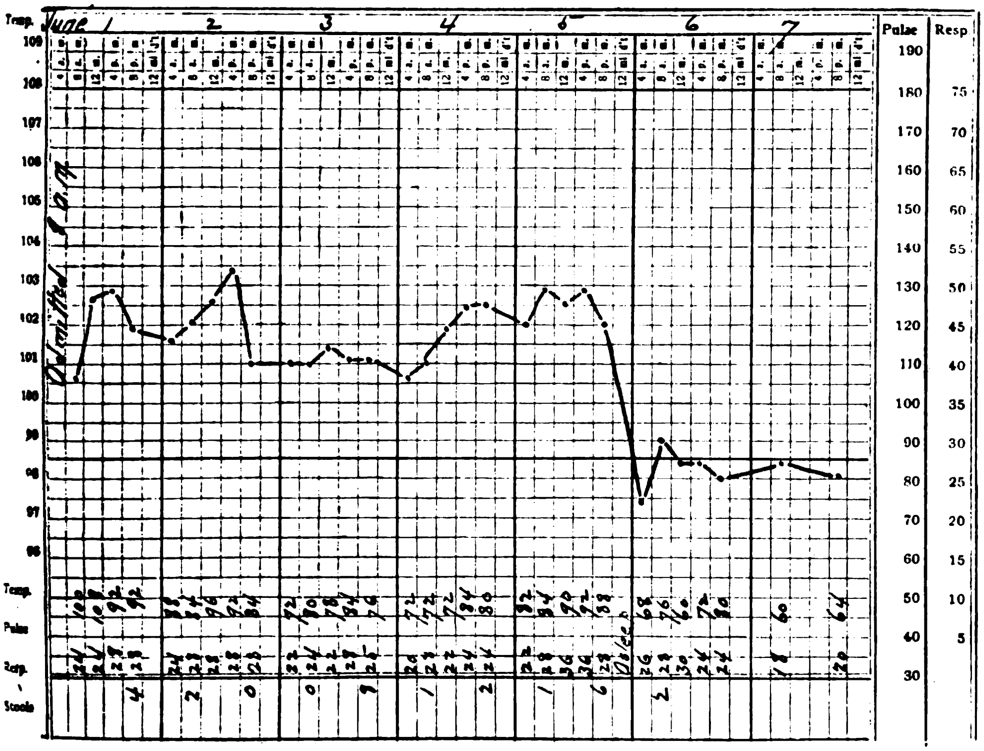
The nature of the infection is not known.



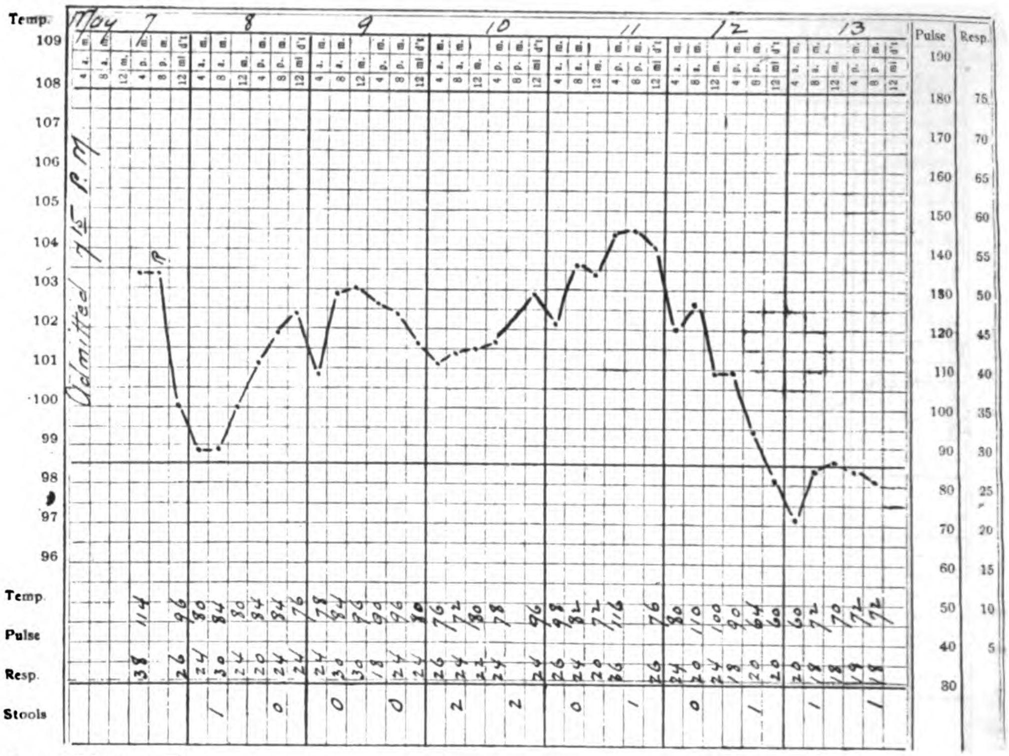
Day of Disease : First.



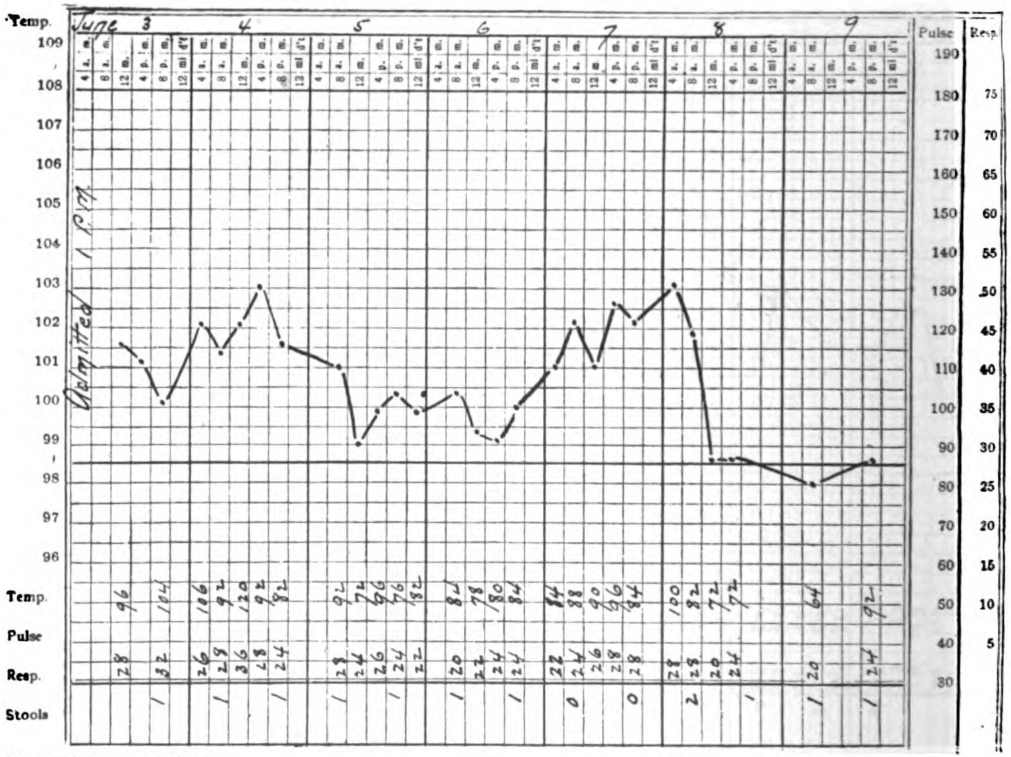
Day of Disease : First.



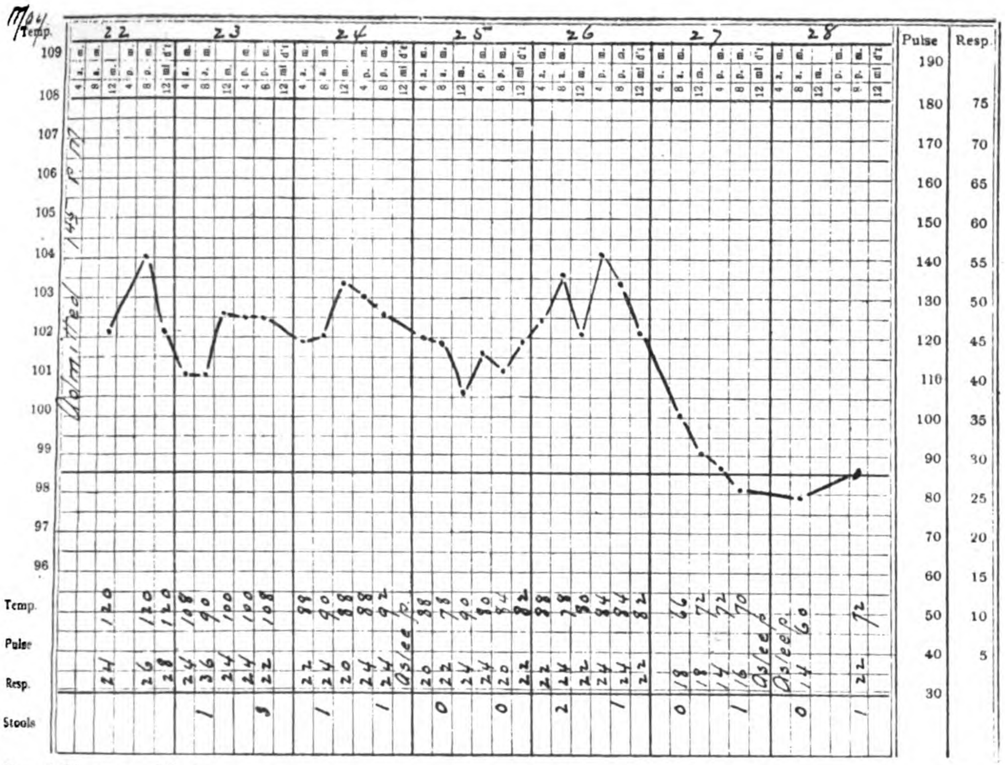
Day of Disease : Second.



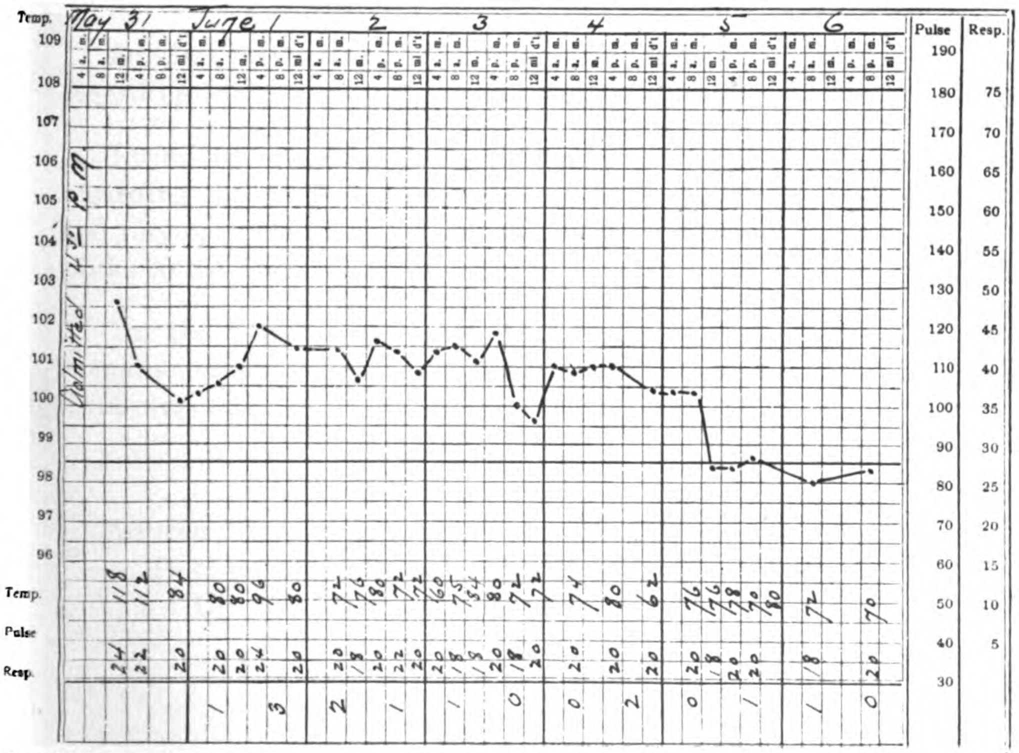
Day of Disease : First.



Day of Disease : First.



Day of Disease : First.



Day of Disease : First.

COMMUNICATION FROM THE COMMISSION FOR THE STUDY OF MALARIA IN RUSSIA

DEAR SIR,

The Commission to study malaria in Russia by the Society of Russian Medical Men, to the memory of N. L. Pirogoff, begs you to print the present letter in your well-known journal.

The Commission to study malaria in Russia by the Society of Russian Medical Men to the memory of N. L. Pirogoff is finishing the index of Russian literature about malaria to 1913, inclusive.

In future the indexes will be issued yearly, with short reports of the articles, including, if possible, all the literature relating to malaria for the past year.

Besides, the Commission will soon edit books of information regarding leishmaniasis and other protozoal illnesses.

Therefore, the Commission addresses the authors of articles regarding the denoted branches of medicine, veterinary medicine and phytopathology, with a request to send printed copies of their works to the Commission.

To the authors who send two copies of their works the Commission will forward the bibliographic index which they themselves edit.

DR. E. L. MARZINOWSKY.

Address: Moscow,
Hospital of the Emperor Paul I.,
Dr. med. E. L. MARZINOWSKY,
President of the Commission.

Volume VIII December, 1914 No. 3

ANNALS
OF
TROPICAL MEDICINE AND PARASITOLOGY

ISSUED BY
THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Edited by
PROFESSOR J. W. W. STEPHENS, M.D. Cantab., D.P.H.
PROFESSOR R. NEWSTEAD, M.Sc., J.P., F.R.S., A.L.S., F.E.S., Hon. F.R.H.S.
PROFESSOR WARRINGTON YORKE, M.D.
AND
PROFESSOR SIR RONALD ROSS, K.C.B., F.R.S., M.D., F.R.C.S.,
MAJOR I.M.S. (RET.)

Editorial Secretary
DR. H. B. FANTHAM,
School of Tropical Medicine,
The University,
Liverpool.

C. Tinling & Co., Ltd.
Printers to the University Press of Liverpool
53 Victoria Street

THE INCORPORATED
LIVERPOOL SCHOOL OF TROPICAL MEDICINE

FOUNDED BY SIR ALFRED LEWIS JONES, K.C.M.G.

(Affiliated with the University of Liverpool)

Hon. President: H.R.H. PRINCESS CHRISTIAN OF SCHLESWIG-HOLSTEIN.
Chairman: MR. F. C. DANSON.
Vice-Chairman: Professor R. CATON.
Hon. Vice-Presidents: THE DUKE OF NORTHUMBERLAND, K.G.
THE EARL OF DERBY, G.C.V.O.
EARL CROMER, G.C.B.
VISCOUNT MILNER, G.C.B.
LORD PIRRIE, K.P.
SIR EDWARD MEREWETHER, K.C.V.O.
SIR OWEN PHILIPPS, K.C.M.G.
SIR WILLIAM H. LEVER, BART.
MR. O. HARRISON WILLIAMS

COMMITTEE

Mr. H. J. READ, C.M.G.	*Colonial Office*
Vice-Chancellor SIR ALFRED DALE	*University of Liverpool*
Sir W. B. BOWRING, Bart.	*Council of University of Liverpool*
Professor R. CATON	
Professor W. HERDMAN, F.R.S.	*Senate of University of Liverpool*
Professor J. M. BEATTIE	
Dr. C. J. MACALISTER	*Royal Southern Hospital*
Mr. G. P. NEWBOLT	
Mr. C. BOOTH (Jun.)	*Steamship Owners' Association*
Mr. T. F. HARRISON	
Mr. A. R. MARSHALL	*Shipowners' Association*
Mr. W. ROBERTS	
Mr. J. W. ALSOP	
Mr. G. BROCKLEHURST	
Mr. C. LIVINGSTON	
Mr. J. W. W. DANSON	
Professor E. W. HOPE	
Mr. R. RANKIN	
Professor E. GLYNN	
Mr. E. G. BUCKLEY	
Mr. A. L. REA, *Hon. Treasurer*	
Mr. A. H. MILNE, C.M.G., *Secretary.*	

B 10 Exchange Buildings, Liverpool

Staff, 1914

1. At the University of Liverpool

Professors - -	JOHN WILLIAM WATSON STEPHENS, M.D., Cantab., D.P.H. *Sir Alfred Jones Professor of Tropical Medicine*
	ROBERT NEWSTEAD, F.R.S., M.Sc., A.L.S., F.E.S., *Dutton Memorial Professor of Entomology*
	MAJOR SIR RONALD ROSS, K.C.B., F.R.S., LL.D., F.R.C.S., D.P.H., M.D., D.Sc. Nobel Laureate 1902. (Indian Medical Service, retired). *Professor of Tropical Sanitation*
	WARRINGTON YORKE, M.D., *Walter Myers Professor in Parasitology*
Lecturers - -	HAROLD BENJAMIN FANTHAM, D.Sc., B.A., *Lecturer in Parasitology*
	HENRY FRANCIS CARTER, S.E.A.C. DIPL. F.E.S., *Lecturer in Entomology*
	WILLIAM THOMAS PROUT, M.B., C.M.G., *Lecturer on Tropical Sanitation*
	HAROLD WOLFERSTAN THOMAS, M.D., C.M.
	PROF. E. W. HOPE, M.D., D.Sc., *Lecturer on Municipal Sanitation*
Honorary Lecturer -	MAJOR JOSEPH FITZGERALD BLOOD, M.D., M.Ch. (Indian Medical Service, retired)
Honorary Statistician	WALTER STOTT
Bibliographer - -	WALTER DRAWZ

2. At the Yellow Fever Bureau

HARALD SEIDELIN, M.D. (Scientific Secretary).

3. At the Royal Southern Hospital, Liverpool

Physicians - -	CHARLES JOHN MACALISTER, M.D., F.R.C.P.
	JOHN LLOYD ROBERTS, M.D., M.R.C.P.
Surgeons - - -	D. DOUGLAS CRAWFORD, F.R.C.S.
	ROBERT JONES, F.R.C.S., Ch.M.
	GEORGE PALMERSTON NEWBOLT, M.B., F.R.C.S.
Tropical Pathologist -	JOHN WILLIAM WATSON STEPHENS, M.D. Cantab.

4. On Expedition

HAROLD WOLFERSTAN THOMAS, M.D., C.M.

WARRINGTON YORKE, M.D.

B. BLACKLOCK, M.D., D.P.H.

Laboratory

JOHNSTON LABORATORY, UNIVERSITY OF LIVERPOOL

Hospitals :

ROYAL SOUTHERN HOSPITAL AND ROYAL INFIRMARY, LIVERPOOL

Secretary's Office :

B 10, EXCHANGE BUILDINGS, LIVERPOOL

NOTICE

The following courses of instruction will be given by the Liverpool School of Tropical Medicine during 1915 :—

Full Course begins 6 January. Advanced Course begins 1 June.
Diploma Examination, 29 March. Certificate Examination, 30 June.
Full Course begins 15 September.
Diploma Examination, 13 December.
These dates are subject to revision.

The full Course of Instruction is open to all qualified medical men, and the Examination to all students who have taken out this full course.

Fee for the full Course of Instruction—Thirteen Guineas.

Fee for the Diploma Examination—Five Guineas.

Fee for the Short Course of Instruction—Four Guineas.

Fee for the use of a School microscope during one term—Ten shillings and sixpence.

For prospectus and further information, application should be made to the Dean of the Medical Faculty, University of Liverpool.

The following have obtained the Diploma in Tropical Medicine of the University of Liverpool:—

Diploma in Tropical Medicine

Date of Diploma	
1904	**Augustine, Henry Joshua**
1904	**Bennett, Arthur King**
1904	**Bruce, William James**
1904	**Byrne, John Scott**
1904	**Clayton, Thomas Morrison**
1904	**Dalziel, John McEwen**
1904	**Dee, Peter**
1904	**Greenidge, Oliver Campbell**
1904	**Hehir, Patrick**
1904	**Khan, Saiduzzafor**
1904	**Laurie, Robert**
1904	**Maclurkin, Alfred Robert**
1904	**McConnell, Robert Ernest**
1904	**Nicholson, James Edward**
1904	**Philipson, Nicholas**
1904	**Sharman, Eric Harding**
1904	**Thomson, Frank Wyville**
1904	**Walker, George Francis Clegg**
1905	**Anderson, Catherine Elmslie**
1905	**Brown, Alexander**
1905	**Caldwell, Thomas Cathcart**
1905	**Critien, Attilio**
1905	**Hooton, Alfred**
1905	**Hudson, Charles Tilson**
1905	**Illington, Edmund Moritz**
1905	**Macfarlane, Robert Maxwell**
1905	**Maddock, Edward Cecil Gordon**
1905	**Moore, James Jackson**
1905	**Nightingale, Samuel Shore**
1905	**Radcliffe, Percy Alexander Hurst**
1905	**Young, John Cameron**
1906	**Adie, Joseph Rosamond**
1906	**Arnold, Frank Arthur**
1906	**Bate, John Brabant**
1906	**Bennetts, Harold Graves**
1906	**Carter, Robert Markham**
1906	**Chisholm, James Alexander**
1906	**Clements, Robert William**
1906	**Dundas, James**
1906	**Faichnie, Norman**
1906	**Jeffreys, Herbert Castelman**
1906	**Mackenzie, Donald Francis**
1906	**Pailthorpe, Mary Elizabeth**
1906	**Palmer, Harold Thornbury**
1906	**Pearse, Albert**
1906	**Sampey, Alexander William**
1906	**Smithson, Arthur Ernest**
1906	**Taylor, Joseph van Someron**
1906	**Taylor, William Irwin**
1906	**Tynan, Edward Joseph**

Date of Diploma	
1906	Watson, Cecil Francis
1906	Willcocks, Roger Durant
1906	Williamson, George Alexander
1907	Allan, Alexander Smith
1907	Allwood, James Aldred
1907	Bond, Ashton
1907	Branch, Stanley
1907	Collinson, Walter Julius
1907	Davey, John Bernard
1907	Donaldson, Anson Scott
1907	Fell, Matthew Henry Gregson
1907	Gann, Thomas William Francis
1907	Graham, James Drummond
1907	Hiscock, Robert Carroll
1907	Keane, Joseph Gerald
1907	Kennan, Richard Henry
1907	Kenrick, William Hamilton
1907	Le Fanu, George Ernest Hugh
1907	Mackey, Charles
1907	Maddox, Ralph Henry
1907	McCarthy, John McDonald
1907	Raikes, Cuthbert Taunton
1907	Ryan, Joseph Charles
1907	Vallance, Hugh
1908	Caverhill, Austin Mack
1908	Crawford, Gilbert Stewart
1908	Dalal, Kaikhusroo Rustomji
1908	Dansey-Browning, George
1908	Davidson, James
1908	Dickson, John Rhodes
1908	Dowdall, Arthur Melville
1908	Glover, Henry Joseph
1908	Greaves, Francis Wood
1908	Goodbody, Cecil Maurice
1908	Harrison, James Herbert Hugh
1908	Joshi, Lemuel Lucas
1908	Le Fanu, Cecil Vivian
1908	Luethgen, Carl Wilhelm Ludwig
1908	Mama, Jamshed Byramji
1908	McCay, Frederick William
1908	McLellan, Samuel Wilson
1908	Pearce, Charles Ross
1908	Schoorel, Alexander Frederik
1908	Smith, John Macgregor
1908	Stewart, George Edward
1908	Tate, Gerald William
1908	Whyte, Robert
1909	Abercrombie, Rudolph George
1909	Allin, John Richard Percy
1909	Armstrong, Edward Randolph
1909	Barrow, Harold Percy Waller
1909	Beatty, Guy
1909	Carr-White, Percy
1909	Chevallier, Claude Lionel
1909	Clark, William Scott
1909	Cope, Ricardo
1909	Fleming, William
1909	Hanschell, Hother McCormick
1909	Hayward, William Davey
1909	Henry, Sydney Alexander
1909	Innes, Francis Alexander
1909	Jackson, Arthur Frame
1909	Kaka, Sorabji Manekji
1909	McCabe-Dallas, Alfred Alexander Donald
1909	Meldrum, William Percy
1909	Murphy, John Cullinan
1909	Samuel, Mysore Gnananandaraju
1909	Shroff, Kawasjee Byramjee
1909	Thornely, Michael Harris
1909	Turkhud, Violet Ackroyd
1909	Webb, William Spinks
1909	Yen, Fu-Chun
1910	Brabazon, Edward
1910	Castellino, Louis
1910	Caulcrick, James Akilade
1910	Dowden, Richard
1910	Haigh, William Edwin
1910	Hamilton, Henry Fleming
1910	Hefferman, William St. Michael
1910	Hipwell, Abraham
1910	Homer, Jonathan
1910	Houston, William Mitchell
1910	James, William Robert Wallace
1910	Johnstone, David Patrick
1910	Korke, Vishnu Tatyaji
1910	Macdonald, Angus Graham
1910	Macfie, John Wm. Scott
1910	Manuk, Mack Walter
1910	Murison, Cecil Charles
1910	Nanavati, Kishavlal Balabhai
1910	Nauss, Ralph Welty
1910	Oakley, Philip Douglas
1910	Pratt, Ishmael Charles
1910	Sabastian, Thiruchelvam
1910	Shaw, Hugh Thomas
1910	Sieger, Edward Louis
1910	Sousa, Pascal John de
1910	Souza, Antonio Bernardo de
1910	Waterhouse, John Howard
1910	White, Maurice Forbes
1911	Blacklock, Breadalbane
1911	Brown, Frederick Forrest
1911	Chand, Diwan Jai
1911	Holmes, John Morgan
1911	Ievers, Charles Langley
1911	Iles, Charles Cochrane
1911	Ingram, Alexander
1911	Kirkwood, Thomas
1911	Knowles, Benjamin
1911	Liddle, George Marcus Berkeley
1911	Lomas, Emanuel Kenworthy
1911	Mackarell, William Wright
1911	MacKnight, Dundas Simpson
1911	Mascarenhas, Joseph Victor
1911	Murray, Ronald Roderick
1911	Oluwole, Akidiya Ladapo
1911	Rao, Koka Ahobala
1911	Sinton, John Alexander
1911	Tarapurvalla, Byramji Shavakshah
1911	Taylor, John Archibald
1911	Woods, William Medlicott

Date of Diploma	
1912	Aeria, Joseph Reginald
1912	Anderson, Edmund Litchfield
1912	Borle, James
1912	Bowie, John Tait
1912	Brassey, Laurence Percival
1912	Christie, David
1912	Dillon, Henry de Courcy
1912	Dunn, Lillie Eleanor
1912	Hardwicke, Charles
1912	Jagose, Jamshed Rustomji
1912	Kochhar, Mela Ram
1912	McGusty, Victor William Tighe
1912	Milne, Arthur James
1912	Mitra, Manmatha Nath
1912	Myles, Charles Duncan
1912	Pelly, Huntly Nevins
1912	Prasad, Bindeshwari
1912	Prentice, George
1912	Ross, Frank
1912	Russell, Alexander James Hutchison
1912	Ruthven, Morton Wood
1912	Sandilands, John
1912	Seddon, Harold
1912	Smalley, James
1912	Strickland, Percy Charles Hutchison
1912	Watson, William Russel
1913	Austin, Charles Miller
1913	Banker, Shiavux Sorabji
1913	Becker, Johann Gerhardus
1913	Carrasco, Milton
1913	Clark, James McKillican
1913	Forsyth, Charles
1913	Grahame, Malcolm Claude Russell
1913	Grieve, Kelburne King
1913	Hargreaves, Alfred Ridley
1913	Hepper, Evelyn Charles
1913	Hiranand, Pandit
1913	Jackson, Oswald Egbert
1913	Khaw, Ignatius Oo Kek
1913	MacKelvie, Maxwell
1913	MacKinnon, John MacPhail
1913	Macmillan, Robert James Alan
1913	Mouat-Biggs, Charles Edward Forbes
1913	Noronha, John Carmel
1913	O'Connor, Edward
1913	Olubomi-Beckley, Emanuel
1913	Pestonji, Ardeshir Behramshah
1913	Puttanna, Dodballapur Sivappa
1913	Reford, John Hope
1913	Smith, Edward Arthur
1913	Stewart, Samuel Dudley
1913	Walker, Frederick Dearden
1913	Wilbe, Ernest Edward
1913	Wilson, Hubert Francis
1913	Yin, Ulg Ba
1913	Young, William Alexander
1914	Arculli, Hassan el
1914	Chohan, Noormahomed Kasembha
1914	Connell, Harry Bertram
1914	Gwynne, Joseph Robert
1914	Hodkinson, Samuel Paterson
1914	Jackson, Arthur Ivan
1914	Kaushash, Ram Chander
1914	Kelsall, Charles
1914	Luanco y Cuenca, Maximino
1914	Rowe, John Joseph Stephen
1914	Sur, Sachindra Nath
1914	Wilkinson, Arthur Geden

EDITORIAL NOTICE

By order of the Committee of the Incorporated Liverpool School of Tropical Medicine, the series of the Reports of the School, which had been issued since 1899, were followed, from January 1, 1907, by the Annals of Tropical Medicine and Parasitology, of which this is the third number of the eighth volume.

Altogether twenty-one Memoirs, besides other works, were published by the School since 1899, and of these ten, containing 519 quarto or octavo pages and 95 plates and figures, were published during the two years 1904 and 1905.

The Annals are issued by the Committee of the School, and will contain all such matter as was formerly printed in the Reports—that is to say, accounts of the various expeditions of the School and of the scientific work done in its laboratories at the University of Liverpool and at Runcorn. In addition, however, to School work, original articles from outside on any subject connected with Tropical Medicine and Hygiene, and Parasitology may be published if found suitable (see notice on back of cover); so that, in all probability, not less than four numbers of the Annals will be issued annually. Each number will be brought out when material sufficient for it has been accumulated.

A STUDY OF SOME CASES OF YELLOW FEVER IN GUAYAQUIL, ECUADOR

BY

J. M. O'BRIEN, M.R.C.S., L.R.C.P.

(*Received for publication* 11 *May*, 1914)

PLATE XVIII

The original purpose of my visit to Guayaquil was to gain some skill in the diagnosis of yellow fever in mild cases and in the early stage of the disease.

Unfortunately, as the dry season prevailed, and as my stay was limited, only twenty cases came under my observation.

Of the really mild cases which I wished most to see there were only two. Probably most of such cases occurring in the town recovered at home, either untreated or treated and diagnosed as malaria.

Those patients that came under my notice were, therefore, suffering from the disease in an advanced or grave form leaving as a rule no possible doubt as to the diagnosis, and according in character with the text-book descriptions. Nevertheless, I would like to bring forward some points which, as far as I know, have not been previously mentioned, or on which little stress is laid.

A slight abnormality of the heart sounds is common. This was brought to my notice by some local practitioners, and I was enabled to corroborate their observations in several cases. The abnormality consists of an alteration of the first sound, sometimes merely a lengthening of it, sometimes merely an alteration of its tone. At other times there is a definite murmur. The murmur may be pre-systolic, systolic or post-systolic in time, generally pre-systolic, and is of a blowing character. It is best heard between the third and fourth costal cartilages, and internal to the nipple line. It appears early in the disease (about the third day), and may disappear at any time subsequently without reference to the condition of the patient.

Of the yellow fever cases that died while I was in Guayaquil, two were subjected to post-mortem examination. In one of these, the aortic valve showed what appeared to be a penetrating ulcer of one of its cusps, while the tricuspid showed some reddening and thickening. In the second case, the mitral valve showed a slight sub-endocardial haemorrhage on the ventricular surface of one of its flaps.

The former case I saw for the first time the day before death, and no murmur could be made out in the very weak beating heart.

The second had a slight pre-systolic murmur on the third day of the disease. This disappeared on the fourth day, death occurring on the morning of the sixth.

It is not improbable that sub-endocardial haemorrhages are the usual cause of this symptom.

I have seen some cases which showed that icteric tingeing of the eyes and skin could be very slight and transient in well-defined cases of yellow fever. Often it does not become at all marked till about the eighth day.

This point has some importance when the patient has, even in health, yellow conjunctiva, combined with the black skin of the coloured races. If one is to use the symptom in diagnosis, a sharp observation of the condition of the eye must be made daily, and it should be borne in mind that the absence of observable increase in the icterus does not exclude yellow fever.

The liver is generally painful. In some cases this pain may be merely an extension of the epigastralgia. In others, however, it extends well round to the side, and occasionally it is limited to particular points.

The spleen is sometimes tender.

In the cases which came under my notice, the patients very rarely complained of rachialgia, when they did, it passed off early in the illness. This is noteworthy if it is remembered that at one time yellow fever was known as '*coup de barre*,' and it suggests that in some epidemics symptoms disappear, which in others have dominated the illness.

An increase in the percentage of albumen in the urine as the temperature drops is one of the classical signs of yellow fever. It is, however, not constant.

Blood examinations

Differential leucocyte counts present the following points of interest.

The percentage of polymorphonuclears is nearly always high.

The number of large mononuclears is generally normal.

The lymphocytes sometimes diminish, almost to disappearing point, but on the other hand, in some well-defined cases of yellow fever, maintain their percentage normally high.

Eosinophiles are frequently entirely absent, but the same may be said of the blood of natives of Ecuador in other diseases.

The percentage of transitionals varies between the usual limits in my counts.

Mast cells are commonly present.

The differential counts in general suggest an increase in the percentage of polymorphonuclears, rather than a decrease in the other elements.

With regard to the occasional very low percentage of lymphocytes, judging from the small number of slides I have been able to examine, I think that a low lymphocyte count is normal to the people of this country.

As convalescence progresses, the percentages adjust themselves to the normal.

That, however, which calls for most attention in these examinations of stained blood (Giemsa) is the condition of the polymorphonuclears.

From what I have recently seen, I am inclined to think that in a very large proportion of yellow fever cases these cells are acutely degenerated. I think that this degeneration is often so considerable on the fourth day of disease as to be of diagnostic value. Since I first observed this phenomenon, I have looked for it in some dozen cases of plague, in a few cases each of dysentery, typhoid, malaria and pregnancy. In all these some few degenerated polymorphonuclears may be seen, but they are not affected in the wholesale manner which can be seen in yellow fever.

In two other cases of fever I saw the first stage of the degeneration. A diagnosis of yellow fever could not be made—nor was any other diagnosis—both patients were non-immunes.

In a typical case, about the third day of the fever, some half of the polymorphonuclears lose the brown stippled staining reaction which their protoplasm has towards Giemsa; by the fourth day almost all have lost this colouring, only faint dots may be seen in the cytoplasm of some, in others the cytoplasm is obviously unstained and hardly discernible.

The nucleus during this stage remains normal.

In the next stage, the protoplasmic edge looks torn and ragged, the nucleus becomes splayed out and lightly stained, and without having seen the various stages of the transformation it would sometimes be difficult to recognise the cell.

Later the protoplasm contracts, assumes a rounded form and takes on a light pink colour. The nucleus is rounded and small, its stain becomes intense.

In the most advanced stage observed the nucleus is round, with perhaps one or two drop-shaped fragments of nuclear matter near it. The cytoplasm is circular, and about twice the size of a red blood corpuscle. It has a pinkish stain. More than anything else it suggests in shape a small round *Amoeba coli.* This form is sparsely scattered in the slides.

This alteration in these corpuscles, which I consider to be a degeneration, largely on account of the loss of staining reaction of the cytoplasm in the first stage, does not always proceed even to the second stage; indeed occasional leucocytes in the third stage are more common late in the disease, than are those in the second stage in the middle term of the disease. On the other hand, so advanced is the second stage sometimes, that it is a matter of surprise that the patient can live with almost every polymorphonuclear leucocyte in such a state of ruin as to be practically unrecognisable. Nevertheless, some of these cases make a quick recovery.

Dr. H. C. Ross, by his staining of leucocytes in life, has given the clue to the condition found in the last stage described. These cells are dead, and their condition can be recognised immediately by persons practised in the use of the jelly method.

In his paper entitled 'Some comparative measurements of the lives of leucocytes (polymorphonuclears) when the cells are resting in the plasmata of different persons' (*Lancet*, Feb. 6, 1909), Dr. Ross says 'Moreover the dead cells may be achromatic, in which case they will not stain. Their nuclei may appear as a single nuclear mass, or their nuclei may even stain.'

The earlier stages described may be states of degeneration preceding death.

I lay some stress on these observations, as I do not know that they have been previously recorded. Yet when once noticed they seem obvious.

In those cases of plague which I examined, the tendency of the polymorphonuclears was to be small, of abnormal shape and with the cytoplasm more intensely stippled than usual. In one case of pneumonia the cytoplasm was large in amount, with coarse, loosely scattered brown granules.

Vacuolation of the polymorphonuclears is common in yellow fever, but not constant. The vacuoles are small and from one to eight in number as a rule. I suggest that they are due to phagocytosis of the blood platelets, their size is in agreement with this suggestion, and phagocytosis of blood platelets may be observed in some cases.

The vacuolation affects the cytoplasm and not the nucleus. It is sometimes responsible for the very tattered appearance of the cells in the second stage of degeneration.

It would be of great interest to ascertain whether those diseases with which mild cases of yellow fever can be easily confused, e.g., Weil's disease, pappataci fever, three days' fever, six days' fever, seven days' fever, pseudo dengue, etc., also show any parallel changes in the polymorphonuclears.

For some time I made a patient search for *Paraplasma flavigenum*, but without success, its very minute size may have allowed it to escape my vision.

IMMUNITY OF INFANTS AND VERY YOUNG CHILDREN

The absence of yellow fever amongst very young children has been frequently observed.

In the records of the children's wards of the Asilo Mann, I find that in 618 children at the breast admitted between October, 1909, and the end of December, 1913, there were no cases of yellow fever.

From January 4th, 1911, to the end of December, 1913, 1,480 boys were admitted, amongst them 24 cases of yellow fever. The average age was nine years and a fraction. One was four years old. Three were five years old. The eldest was fifteen years old (although children over twelve are not usually admitted) and a native of Guayaquil, as was one other of eleven years of age.

2,218 girls were admitted between January, 1907, and the end of December, 1913, amongst these were 19 cases of yellow fever. This is a notably lower percentage than that given by the boys. The average age in these nineteen cases was eight and a half. One was four years old, and two were five years old. Three were born in Guayaquil, and were respectively five, six and seven years of age when attacked with yellow fever.

I have spoken on this subject to many medical men in Guayaquil.

These practitioners are as well acquainted with yellow fever as ours of London are with broncho-pneumonia or measles, yet they are unanimous in stating that they have never seen the disease in infants. It may, therefore, be granted that infants and very young children are endowed with an immunity; not only the children of those who have suffered from the disease, but all children, for many of those which would come under medical care in Guayaquil are serranos—their parents poor labouring people from the mountains, who form a considerable proportion of the floating population of the town.

The suggestion that children are gradually immunised by being frequently bitten by infected mosquitos may be immediately put aside, for other things being equal, would not adults have at least as good a chance of acquiring such immunity.

This immunity is a thing which gradually wears off, as is evidenced by the fact that the number of cases amongst children increases with age.

The small number of figures collected by me in the records of the Asilo Mann may be arranged as follows:—

Ages	4	5	6	7	8	9	10	11	12
Number of Cases....	2	5	3	5	3	6	7	9	3

(Many children of 12 years would go to the General Hospital.)

If children are born with absolute immunity, which they gradually lose, it is easy to understand how they acquire a permanent immunity by continually living exposed to infection. As they lose 'infancy immunity' they are subject to one or more slight undiagnosable attacks of fever, i.e., they receive doses of the virus which would in a non-immune adult produce grave illness, but which their remaining partial immunity largely neutralizes. Thus by these gentle vaccinations they acquire a lifelong protection.

Two cases interesting in this way were brought to my attention.

Two girls, of German parentage, born in Guayaquil, had not suffered from yellow fever till they were respectively four and five years of age. At this time they were sent to Germany, and remained there for six years.

Within a few months of their return, both suffered from definite attacks of yellow fever. During their earlier childhood the amount of yellow fever present in Guayaquil was similar to that existing on their return. Their 'infancy immunity' protected them during their earlier residence in the endemic area. During their stay in Europe this wore off, and they had not had sufficient opportunity to establish a perfect 'life immunity.'

How do children come to have this very peculiar immunity to a certain disease?

Dr. Pareja, of Guayaquil, suggested to me that as the liver in children is larger in proportion to the body than is the case in adults, it may have some function or secretion absent in the adult, and which has the power of combating the yellow fever virus. This suggestion is useful, and might be applied to other organs of the body. The difficulty of experiment in the matter is obvious, but if this property of children is common to other young mammals, it might have its use either as a protective or as a curative agent.

In speaking here of *immunity* to the *disease*, I do not exclude a possible *tolerance* to the *parasite*.

It is conceivable that children subject to frequent bites of infected mosquitos do allow the parasite to remain alive for a time within the body. A parallel to this may be seen in the tolerance of some negroes to malarial infection.

TREATMENT

Treatment limits itself to purgation combined with occasional bleeding in the early and congestive stages of the fever, alkaline waters (e.g., Vichy) to counteract the lessened alkalinity of the blood, alkaline draughts, champagne, and ice to allay the vomiting, injection of artificial serum in collapse from loss of blood, vomiting, and in acute oliguria, and anuria; in the latter case aided by a variety of diuretics, especially Teocine.

Digitalis, caffeine, injections of camphor oil, and strychnine are given as cardiac stimulants.

Cold or tepid baths are administered about four times a day in some cases, and it is said with good results. Hot fomentations may somewhat remove the abdominal pains.

All these will at times give relief and aid the patient to recovery, but nothing alters definitely the course of the disease.

It is stated that the coagulability of the blood is much lowered in yellow fever. It may be that this is caused by the phagocytosis of blood platelets which is sometimes seen to occur. To increase the coagulability calcium chloride has been administered by the mouth and intravenously, but without any recognisable action.

DIAGNOSIS

The classical symptoms of yellow fever may be given as follows:—Pyrexia, early congestion, bradycardia albuminuria, increase of albuminuria with fall of temperature, oliguria, late icterus, and black vomit. Of these, pyrexia and albuminuria are the only ones that show any constancy. Had we some fine laboratory test for the disease, it might be that in the mildest cases albuminuria would also be sometimes absent. This inconstancy renders certain diagnosis in many cases impossible. One must be dealing with a possible non-immune who has been liable to infection, then eliminate other diseases. If, after this, we have any four of the above signs left, the case should, from a sanitary point of view, be considered as yellow fever.

EXPLANATION OF PLATE XVIII

The specimens were all stained with Giemsa. The time of staining varied from ten minutes to one hour.

Fig. 1. Normal polynuclear leucocyte for comparison.

Fig. 2. Transition from first to second stage.

Fig. 3. Advanced second stage, every polymorphonuclear in film affected, a condition resembling that produced by haemolysis.

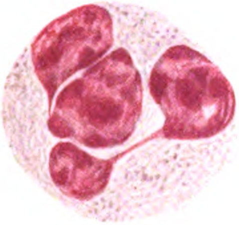
Fig. 4. Transition from second to third stage.

Fig. 5. Early third stage.

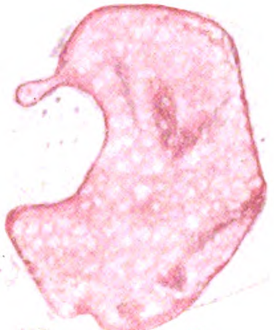
Fig. 6. Third stage.

Fig. 7. Third stage.

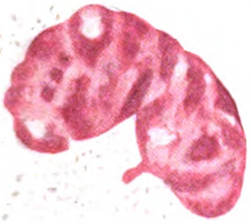
Figs. 8, 9, 10. Advanced third stage.



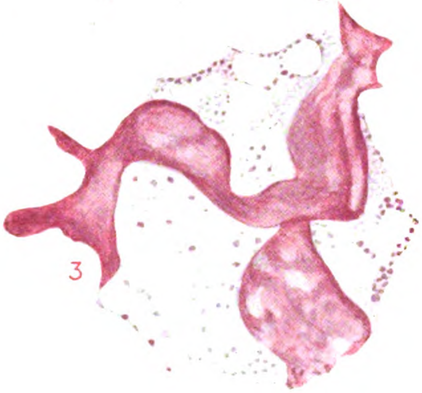
1



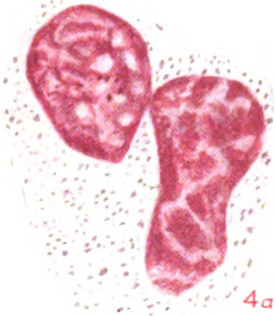
2a



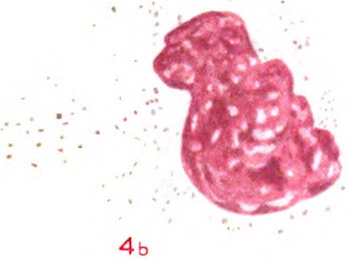
2b



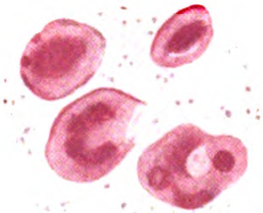
3



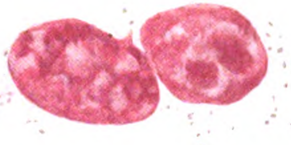
4a



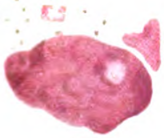
4b



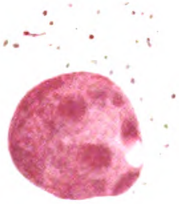
5



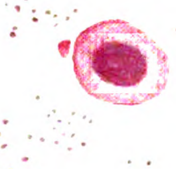
6



7



8



9

10

P. P. Press, Imp.

SLEEPING SICKNESS IN THE EKET DISTRICT OF NIGERIA

BY

J. W. SCOTT MACFIE, M.A., M.B.

AND

G. H. GALLAGHER, L.R.C.S., L.R.C.P.

WEST AFRICAN MEDICAL STAFF, NIGERIA.

(*Received for publication* 14 *April*, 1914)

PLATES XIX—XXIII, AND MAP

Although the Eket district is by no means one of the least accessible regions of Southern Nigeria, and has been the field for the activities of the Qua Iboe mission for a quarter of a century, it was not until recently that the presence of sleeping sickness was suspected. This fact is no doubt accounted for by the peculiarly mild form assumed by the disease, which at first sight would not suggest the malady which has devastated the Congo and Uganda. Indeed, but for the acuteness of the observation of Mr. W. C. W. Eakin, of the Qua Iboe mission, it might have remained unidentified for many years longer. Eight years ago Mr. Eakin's suspicions were first aroused by the appearance of a sick woman at Ikotobo, and in 1911, another case having come under his notice, he decided that the matter should be reported officially. Mr. S. A. Bill accordingly communicated with the medical officer at Calabar, and early in 1912 Dr. R. W. Gray visited the district for the purpose of investigating the subject. In May Dr. Gray was able to report that he had found trypanosomes in the gland juice of several patients, thus confirming Mr. Eakin's suspicions; and as the disease was found to be prevalent throughout the neighbourhood, it was decided to establish an isolation camp at Ikotobo,* where the patients might undergo treatment.

---

*The name of this town, which is generally pronounced Ikorobo, is more correctly spelt Ikotobo.

At our suggestion Mr. Eakin has very kindly written an account of his observations, which is included as an appendix to this paper (Appendix I). He has also recorded the native views on sleeping sickness in a manner that could only have been done by one who spoke their language, and had won their esteem and trust by long years of devoted service. His account is of considerable interest, and will serve to illustrate how deeply a consciousness of the presence of sleeping sickness has influenced the lives of the people. Some idea of the importance attached to the disease by the natives may be gathered from the fact that their chiefs not only voluntarily gave the land for the camp, but also erected all the buildings, including a house for a medical officer, a hospital and dispensary, and accommodation for about 130 patients.

The task of investigating the disease and starting the isolation camp was entrusted to the late Dr. P. F. Foran. In carrying out this work he displayed untiring energy, and during the year he was in Eket succeeded in winning the confidence of the people. His work can best be judged by its results, and the ready way in which the natives have availed themselves of the advantages afforded them at Ikotobo is eloquent testimony to his success. But for his untimely death, which occurred on the voyage back to England, he would no doubt have written an account of sleeping sickness as he found it in the Eket district. This unfortunately is now impossible. We believe, however, that a description of the disease, which presents certain unusual features, will be of interest; and although we cannot hope to give as complete an account as could have been given by Dr. Foran, we take this opportunity of expressing our indebtedness to his careful investigation, and our deep regret that he was unable himself to record the results of his observations.

PHYSICAL AND OTHER FEATURES OF THE EKET DISTRICT

The Eket district, a division of the Eastern province of Southern Nigeria, occupies the tongue of land lying between Calabar and Opobo. It is situated just north of 4° 30′ North latitude, and is divided into two unequal portions by the 8th degree of East longitude. To the north it is bounded by the districts of Calabar and Uyo, to the west by Opobo, to the south by the sea, and to the east by the Cross River. The area of the district is estimated as 690 square miles (see Map).

The country is well watered by creeks and streams throughout its extent, and in the wet season the southern portions, which are low lying and at other seasons swampy, are under water. This part of the district is forest clad, and except for a few fishing villages on the coast, is practically uninhabited. The Qua Iboe River traverses the western portion of the district on its way to empty itself into the sea, and is joined by the Obium River (Pl. XIX, fig. 2) just above the town of Eket, the headquarters of the district. The Obium River drains the central and south-western portions of Eket, and is a stream of considerable size. The towns and villages on the banks of this river, and of its tributary streams, are those in which sleeping sickness appears to be most prevalent.

The vegetation all over the country is either thick scrub, or forest in which palm trees are abundant. The villages consist of a larger or smaller number of compounds separated, and often hidden from one another by dense bush; so that although they are in fact innumerable, it is possible to pass for considerable distances along the main roads without seeing a single one (Pl. XIX, fig. 1). There are no exceptionally large towns, but rather a great number of small towns and villages. The total population of the district, according to the census of 1911, is estimated at about 184,000—that is the average density of the native population per square mile is 266·64. The chief tribes are in order the Ibibio, the Eket, and the Oron.

The climate, as would be expected, is humid. Meteorological observations have not been recorded, but it may be assumed that they would have been similar to those of Calabar, where the average rainfall is 147·3 inches, the average maximum temperature 88·3° F., the average minimum 71° F., and the mean monthly humidity 88·1.

Sheep, goats, and dogs are common throughout the district, and in most of the towns and villages a few dwarf cattle are to be found. These domestic animals appear to thrive. Larger cattle and horses are unknown. In some places pigs are kept also, but as they are considered unclean animals, and are forbidden by native law, their presence is often concealed. Game is rare in most parts of the district, as might be expected, considering the density of the population. Small antelope are occasionally seen, however, and in the southern areas elephants even may be met with. Monkeys are common in some places, especially in the forests along the river banks.

Biting flies are fairly numerous. Tabanidae are abundant on the Qua Iboe River, and *Chrysops dimidiata* has been taken in several localities. Tsetse flies are widely distributed over the district, but seldom occur in large numbers. During a visit of two months to Ikotobo one of us (J. W. S. M.) never saw a single specimen at liberty, although the house he occupied was situated close to a small stream with thickly-wooded banks that appeared to be an ideal haunt for *Glossina palpalis*, and was within a yard or two of the main road through the district along which a constant procession of natives passed all day. A specially trained collector was employed to catch tsetse flies, but he seldom succeeded in bringing back more than half a dozen as the result of a day's search, and in order to obtain these he had often to go long distances into the bush.

During the months of November and December practically all the tsetse flies caught near Ikotobo were *G. tachinoides*. Only two specimens of *G. palpalis* were taken, and a single example of *G. caliginea*, a male, was obtained from near Eket. *G. tachinoides* is unquestionably the most common species throughout the neighbourhood of Ikotobo, which is at the centre of the sleeping sickness area. The natives are familiar with tsetse flies, and affirm that they are most troublesome in the bush, and not, as might be expected considering the nature of the country, at the waterside. This observation corresponds with the fact that *G. tachinoides* appears to be the predominant species, and that *G. palpalis* is relatively rare. A point of some interest is that *G. tachinoides* is particularly easy to obtain where pigs are found, and is often seen attacking these animals. The natives themselves regard pigs as unclean, and bearing in mind their recognition of the fact that biting flies follow these animals (see Appendix 1), it seems possible that the reason for this belief may have something to do with the association with tsetse flies. The people are not Mohammedans.

In this connexion it is of interest to note that Moiser (1913) in writing of the haunts and habits of *G. tachinoides* in Bornu, Northern Nigeria, states 'Another noticeable feature of all the belts I have examined is the large number of warthogs to be found in and around them.'

G. tachinoides is exceedingly common in Northern Nigeria, but has hitherto been recorded from very few localities in Southern Nigeria. Simpson (1912) mentions only two, Ikom and Bende, but remarks that its occurrence at the latter station 'would seem to indicate that it may be found more commonly in the Cross River basin than is supposed.' In dealing with the distribution of the various species of *Glossina*, the same writer says 'The conditions which are most favourable for *G. palpalis* are, generally speaking, most unfavourable for *G. tachinoides*. Where the country is open, the vegetation sparse, the dry season well defined and the rainfall slight, there *G. tachinoides* is most abundant.' It is somewhat remarkable therefore that *G. tachinoides* should be so common in Eket, where the dense vegetation, the humidity of the atmosphere, and the heavy rainfall would appear to be more suitable for *G. palpalis*. Roubaud (1911), however, while recognising that in Upper Dahomey *G. tachinoides* represents the *G. palpalis* of the more southerly regions, lays emphasis on the fact that both species are hygrophilous, and need the constant humidity of the forest belts, and that it is its resistance to heat alone which enables *G. tachinoides* to overlap northwards the area of *G. palpalis*.

AETIOLOGY OF THE DISEASE

Sleeping sickness has undoubtedly existed in the Eket district for a great number of years. The natives are unanimous in stating that it has been familiar to them all their lives; but some of them assert that during recent years its incidence has increased owing to the neglect by the present generation of the traditional laws and customs relating to the behaviour of infected persons. Whether this is in fact the case cannot be determined with certainty, but that these laws exist is in itself a proof of the antiquity of the disease. Tradition moreover affirms that there was a time, long ago, when the disease was a much more serious scourge than it is at present, when whole villages were exterminated by it.

In the first appendix to this paper a full account is given by Mr. Eakin of the history and traditions derived from native sources. It will be unnecessary to repeat any of this matter since the subject is there dealt with in such an admirable manner.

During the sixteen months in which sleeping sickness has been under investigation, 222 cases have been identified in which the trypanosomes have been demonstrated. In addition 114 cases have been met with presenting some of the clinical features of the disease but in which the parasites have not actually been found. There can be little doubt that the majority of these were actually cases of trypanosomiasis. In what follows no account has, however, been taken of them, only those cases being considered in which the clinical diagnosis has been confirmed by the aid of the microscope. Eighty-four of the cases have been studied by us personally, but for the details of the remainder we have relied on the notes in the case-books at the camp, which for the most part were recorded by Dr. Foran.

Cases have been detected in every part of the district, but a glance at the accompanying map, on which the towns in which sleeping sickness has occurred are specially marked, will show that the disease is most prevalent in the neighbourhood of Ikotobo, and in the areas drained by the Obium River. Beyond the bounds of Eket the occurrence of sleeping sickness has not been investigated. There can be little doubt that the disease will be found to extend to some extent at any rate into the neighbouring districts, but as this fact has not yet been established, and as we are not in a position to state that the disease occurs in an identical form elsewhere, for the purposes of this paper we shall limit our observations strictly to those cases studied at Ikotobo, all of which had come from the Eket district.

THE INFLUENCE OF SEX AND AGE

SEX

Todd and Wolbach (1911) found that in the Gambia the numbers of men and women suffering from sleeping sickness were approximately equal, but that more boys than girls were infected. In the Congo, in 1903-05, the figures were somewhat different. In a recent paper Todd (1913, see Table I) has tabulated the cases examined according to sex and age, and it appears that many more men and boys (63·2 % and 6·7 %) were found to be infected than women and girls (26·8 % and 2·6 %). Sanderson (1912) and Hearsey (1912) in Nyasaland have also recorded a much higher percentage of cases in males than in females, the latter stating that 'the number of males diagnosed as infected is nearly twice that of females.'

In Eket also males appear to be more affected by the disease than females. Of the 222 cases in which the diagnosis has been confirmed by the discovery of trypanosomes, 140 (or 63 %) were males, and 82 (or 37 %) females. As in the Gambia the numbers of men and women were approximately equal (24·3 % and 19·4 % respectively), but many more boys (38·7 %) than girls (17·6 %) were infected.

To what extent these figures represent the actual distribution between the sexes it is difficult to decide. The natives themselves assert that the disease is as common in females as in males. Todd (1913) records that in the Gambia 'glandular enlargement is common among children of both sexes, but especially in boys—probably because boys are less careful of their person than are girls.' As will be pointed out later, the majority of the patients in Eket are children, and it has been mentioned that glandular enlargement is, in the eyes of the natives, almost pathognomonic of the disease. It is possible that boys, if they are really less careful of their persons, may be more liable than girls to the impetigo of the scalp that is so common in Eket, and that this may cause a greater number of them to exhibit enlargement of the cervical glands. This in turn would account for a larger number of boys being brought under observation than girls.

If this is a true explanation it is possible that an even greater number of children may be infected than the statistics show; and that only those cases are brought for treatment, and are thus identified, which from one cause or another have a considerable degree of glandular enlargement.

Another possible explanation suggests itself. Impetigo of the scalp is exceedingly common amongst the children, and in not a few cases, in which a clear history has been elicited, has been stated to have preceded the symptoms of sleeping sickness. It is possible therefore that the affection of the glands following the appearance of the impetigo may enable the trypanosomes to establish themselves in the subject, and that the boys, being less scrupulously clean, may be more liable to this inroad of infection. On the other hand the condition of the scalp and skin may have been secondary to the slight abrasions to which children are subject. For it has been observed by Borchers (1912) that the tissues of patients suffering from sleeping sickness are unable to protect themselves against infection.

AGE

In Tables 1 and 2 the 222 cases of sleeping sickness are distributed according to sex and age in the manner suggested by Todd (1913). In explaining his reasons for adopting these unusual age periods, which are different for the two sexes and do not contain an equal number of years, this author states that they 'were chosen because they represent, approximately, the periods during which male and female natives follow the occupations of children, adults and elderly people.' 'Therefore,' he adds, 'in comparing the figures . . . . . it must be remembered that errors in compilation have occurred, and that the span of years, allotted by the classification employed, is for adults almost thrice that apportioned to children; consequently, a meaning can be attached only to very considerable and constant differences in the figures stated for each class.' We have adopted the same age periods both because we wished to compare our figures with those given by Todd for the Congo and the Gambia; and because the classification, based on occupation, is in our opinion a convenient one. It is often difficult to estimate the age with accuracy, and little assistance is to be obtained from the natives themselves who have but an imperfect conception of the longer intervals of time. Custom, however, arrogates certain offices to the children and the aged, and by bearing these in mind, together with the physical appearances, it is possible to make a fairly accurate guess at the age of every individual. In the graph (Chart 1) showing the distribution of the cases in periods of five years a more exact determination of the ages of the patients is, however, attempted.

TABLE 1.—Analysis of the cases of trypanosomiasis seen in Eket, and a comparison with the figures for the Congo and the Gambia.

SEX	MALE			FEMALE			TOTALS	
Age	0—13	14—44	45—	0—11	12—39	40—	Percentages	Number of cases seen
Congo, 1903-05, selected populations	6·7%	63·2%	0·7%	2·6%	26·8%	0·0%	100%	269
Congo, 1903-05, unselected populations	6·1	57·2	2·0	2·0	32·7	0·0	100	147
Gambia, 1911	16·4	39·3	0·0	7·6	36·7	0·0	100	79
Eket district of Nigeria ...	38·7	23·9	0·4	17·6	19·4	0·0	100	222

TABLE 2.—Analysis of the cases of trypanosomiasis seen in Eket, and a comparison with the figures for the Congo and the Gambia.

Country	AGE			SEX	
	Children	Adults	Aged	Male	Female
Congo, 1903-05, combined populations	8·7%	89·95%	0·35%	67·95%	32·05%
Gambia 1911	24·0	76·0	0·0	55·7	44·3
Eket district of Nigeria	56·3	43·3	0·4	63·0	37·0

On comparing the figures for Eket with those for the Congo and the Gambia there is seen to be one outstanding feature, namely, the very large percentage of the cases that occurred in children. Over 56 % of the cases of trypanosomiasis seen in the Eket district were in children. The corresponding figure for the Gambia is given by Todd as 24 %; and for the Congo 9·3 % or 8·1 %, according as the population from which the cases were drawn was a selected or an unselected one. Such a degree of disparity can hardly be explained by errors of compilation. In Eket, as has

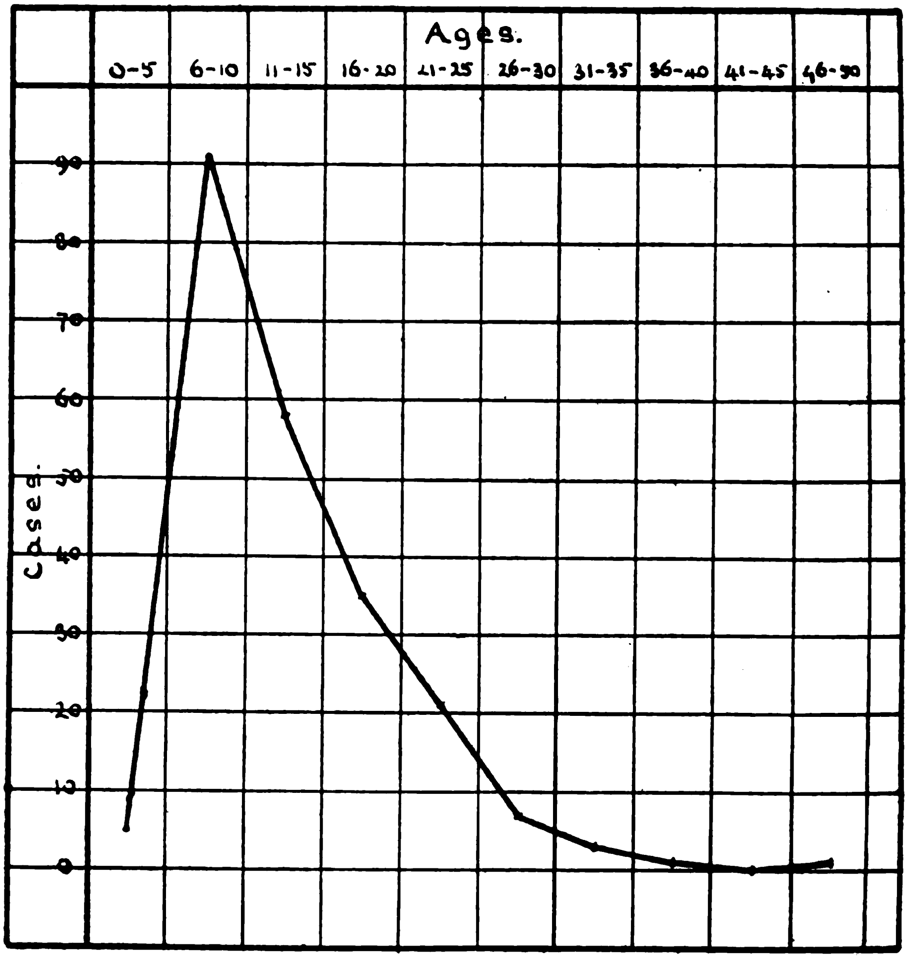


Chart I—Graph showing the ages, grouped in periods of five years, of 222 cases of trypanosomiasis identified in the Eket district of Southern Nigeria in 1912-13.

already been explained, the natives are only too willing to avail themselves of the treatment afforded them at the isolation camp, and seldom offer any objection to examination. Indeed, they are ready to submit even to the excision of a gland for diagnostic purposes, as this form of treatment is that adopted by their own 'medicine men.'

Since natives of all ages and both sexes are accustomed to frequent the camp, there is no reason why the percentage of cases in children should be so high unless the disease is really most prevalent amongst them. It might even have been expected that relatively few children would have been brought for treatment, either from a natural distrust of strangers and foreigners, or because their number was actually small owing to the impotence that is an early symptom of the disease in adults.

Why then should the children be especially affected? One possible explanation may be found in the fact that they are the water carriers and the collectors of firewood, and being mostly unclad, are thus exposed to the attacks of the tsetse flies at the waterside and in the bush (Pl. XXI, fig. 8). Even the infants are not completely protected from the chance of infection since, slung on their mothers' backs, they are carried out into the cultivated lands where *G. tachinoides* is frequently found. Several of the infected adults were collectors of palm-wine, and therefore spent much of their time in the forests or thick bush. But whether they be farmers, traders, or what-not, and irrespective of sex and age, it may be assumed that all are exposed to the attacks of tsetse flies, especially to those of *G. tachinoides*, the species which is not only the most common, but also the frequenter of the bush and the partly cultivated grounds.

Although the majority of the cases were children, the range of age of the patients was considerable. At the time of writing (December 31st, 1913), the youngest inmate of the camp was 2½ years of age, and the oldest 50. A closer analysis shows that 67·1 % of the cases occurred between the ages of 6 and 15 years, and that 85·1 % were in persons under 21.

DIAGNOSIS

Diagnosis has presented unusual difficulties. The majority of the cases brought to the camp have shown very slight symptoms, and would have been unrecognisable clinically. As a rule there was some degree of glandular enlargement, but as many of the children in the native towns have dirty heads, and as in a tropical country there are many causes which may produce this condition, it was impossible to base a diagnosis on this single sign. It is probable that the parents of the patients often observed other symptoms that would not be apparent to strangers, such as the change of character mentioned in the Report of the French Commission (1909). Their diagnosis indeed was seldom at fault. For this reason, and because it was inadvisable that the natives should be discouraged in any way from bringing in cases, a confirmation of the diagnosis by the finding of the parasites was a matter of importance. In practice, however, the trypanosomes were extremely difficult to detect, and in all the cases that came under our notice were rare. Dr. Foran, earlier in the year, noted that in several instances trypanosomes had been numerous in gland juice, a fact that may perhaps be accounted for by the most pronounced cases having been brought under his notice, since he was the first to make a systematic search for them.

The methods of diagnosis employed were the examination of thick and thin blood films, and of gland juice obtained either by puncture or excision of one of the enlarged cervical glands. Centrifugalization of blood and cerebro-spinal fluid could not be employed owing to lack of the necessary apparatus. Inoculation into susceptible animals was only carried out in one or two cases, as experimental animals were difficult to obtain, and because, as has been shown elsewhere, the trypanosome causing the sleeping sickness in Eket has a low degree of virulence, and often fails to infect the most convenient and procurable animals for the purpose, namely guinea-pigs. Monkeys, which might have proved of considerable value, were unobtainable.

By blood examination

Very variable results have been obtained from the examination of the blood in sleeping sickness by workers in different countries.

The members of the French Commission (1909), Martin, Leboeuf and Roubaud, found trypanosomes in 36-45 % of their cases in the French Congo and actually preferred blood examination to gland puncture for rapid diagnosis. Dutton and Todd (1906) in the Belgian Congo found parasites in the blood in 13·6 % of the cases. In the Gambia, however, Todd and Wolbach (1911) found trypanosomes in the blood in only eight out of 362 cases examined by fresh cover-slip preparations, in ten out of 316 examined by thin blood smears, and in eight out of 265 examined by thick blood films. Thiroux and D'Anfreville de la Salle (1911), and more recently Bourret (1913), have drawn attention to the fact that trypanosomes are seldom found by direct examination of a drop of blood in Senegal. The latter observer was successful by this method in only one case out of 45.

Thus the percentage of cases of sleeping sickness in which trypanosomes can be detected by an examination of the blood has been variously stated as from 45 % to 2 %. In the Eket district no case has yet been seen in which trypanosomes were to be found by an examination of a drop of the peripheral blood. Cases have been examined at all stages of the disease, and at different hours of the day, but always with negative results. Both fresh and stained preparations have been used, and thick dehaemoglobinized films have also been tried. Not only has it been our experience that trypanosomes could not be found in the blood by ordinary examination, but it was also that of Dr. Gray, who first identified the disease, and of Dr. Foran who spent a year in the district and collected a large number of cases.

Although blood examination for trypanosomes has been unavailing, the occurrence of auto-agglutination of the red blood corpuscles has been of some assistance in diagnosis. Yorke (1911) who studied this phenomenon concluded that 'a marked degree of auto-agglutination of the red blood cells is an extremely rare occurrence apart from an infection with trypanosomes.' Our observations were perforce made on coverslip preparations, a method of demonstrating the sign which is apparently fallacious. It may be of some interest to note, however, that in practically every case examined in this way auto-agglutination was well marked.

By gland examination

Since Greig and Gray (1904) stated that trypanosomes were almost invariably present in the glands of infected persons, gland puncture has assumed the first place in the routine examination for sleeping sickness. Dutton and Todd (1906) found trypanosomes in 97·2 % of cases by this method, and Koch and his colleagues (1909) in a similarly large percentage. Martin and Leboeuf (1908) were almost as successful, but they noted that as a rule the parasites were rather rare. Although therefore trypanosomes are not invariably to be found, there can be little doubt that in any case in which there was a sufficient enlargement of the cervical glands they would be explored by most observers before attempting any other method of diagnosis. In endemic areas in which the parasites are difficult to detect in the blood this method of examination is indeed the only convenient one to adopt.

All the cases diagnosed in the Eket district were examined in this way. In most of them a posterior cervical gland was punctured, but if no gland in this situation was sufficiently enlarged, one was chosen in the axilla or the epitrochlear region. In our experience trypanosomes were always rare in the gland juice, the heaviest infection met with during the months of November and December being seven parasites in thirty fields, and this was quite an exceptional case. It was often necessary to puncture a number of different glands before finding the parasites.

In many cases that for other reasons were considered highly suspicious of sleeping sickness gland puncture failed to reveal the presence of trypanosomes. In such cases excision of a cervical gland was resorted to. This practice was adopted not only because trypanosomes were sometimes found in the excised gland when they had been absent from gland juice obtained by punctures, but also because the patients were very willing to have this little operation performed, as it was the recognised native treatment for the condition.

Not infrequently, however, examination of the glands, no matter by what means, failed to reveal trypanosomes. More than a hundred cases of this sort have been met with. Clinically they suggested sleeping sickness, and the natives, whose acuteness of observation is remarkable, believed that they were suffering from this disease. In some of them the glands were fibrosed, which may have accounted for the absence of the parasites. One of the cases proved fatal.

GLAND PALPATION

In Eket, since blood examination was fruitless, gland examination had to be relied upon for diagnosis. Centrifugalization of the blood or of the cerebro-spinal fluid could never be of much practical assistance except at headquarters owing to the skill and leisure required for its application; and inoculation into susceptible animals on a scale that would be essential to ensure success could not at present be contemplated. Gland palpation followed by gland puncture would therefore appear to be the most suitable measures to employ for the detection of cases, and for the determination of the areas affected by the disease.

It was not possible in the limited time at our disposal to make a systematic examination throughout the district, but the inhabitants of two selected villages were palpated for enlarged glands in the posterior cervical triangles. The results of these examinations are given below in tabular form (Tables 3 and 4). The first village, Ikot Offiong, was chosen because it was one of the places in which sleeping sickness was first observed, and is a typical example of a place in which the disease is prevalent. The second, Mpok, a town outside the area especially involved, was chosen to represent a village but slightly subjected to the attacks of the disease.

Ikot Offiong (Pl. XIX, fig. 1).

Ikot Offiong, a small village buried in dense bush, is situated near to Ikotobo, in the middle of the sleeping sickness area. It is some distance from the nearest stream, and is surrounded on all sides by trees and scrub. The village itself consists of a number of scattered compounds. Several cases of sleeping sickness have occurred here, and the village was considered to be a highly infected one. Of the 137 people examined, 53 were males and 84 females; the majority were children. Much enlarged (' + ') glands were present in 27·6 % of the boys, and in 17·5 % of the girls; and slightly enlarged (' + − ') glands in 48·3 % and 52·5 % respectively.

Too much reliance should not be placed on these figures since the number of persons examined was small. The most conspicuous feature was the number of children with glandular enlargements of the degrees ' + ' and '.+ – .'

TABLE 3.—The incidence of enlarged posterior cervical glands among 137 natives at *Ikot Offiong* classified according to sex and age.

SEX	MALE			FEMALE		
Age	0—13	14—44	45—	0—11	12—39	40—
' + ' or much enlarged glands	27·6%	0·0%	0·0%	17·5%	0·0%	0·0%
' + — ' or slightly enlarged glands ...	48·3	0·0	0·0	52·5	20·8	20·0
' + — — ' or very slightly enlarged glands	17·2	22·2	16·7	20·0	25·0	20·0
' — ' or normal glands	6·9	77·8	83·3	10.0	54·2	60·0
Totals	100	100	100	100	100	100
Number of individuals of each class examined	29	18	6	40	24	20

Mpok

Mpok is a similar, but larger village. Unlike Ikot Offiong, it is situated to the west of the Qua Iboe River, in a locality where sleeping sickness is now almost unknown. Some years ago, indeed, a native 'doctor' lived in Mpok, and practised excision of glands for sleeping sickness; his patients, however, became so few that he was compelled to remove to another district. Altogether 825 persons were examined, 313 males and 502 females; of these 297 were children. Glandular enlargements of the degrees ' + ' and ' + – ' were much less common than at Ikot Offiong, and the

TABLE 4.—The incidence of enlarged posterior cervical glands among 825 natives at *Mpok* classified according to sex and age.

SEX	MALE			FEMALE		
Age	0—13	14—44	45—	0—11	12—39	40—
' + ' or much enlarged glands	4·0%	0·0%	0·0%	1·7%	0·3%	2·8%
' + — ' or slightly enlarged glands ...	39·2	5·6	0·0	20·3	3·9	2·8
' + — — ' or very slightly enlarged glands	53·6	48·1	46·4	61·6	70·7	55·5
' — ' or normal glands	3·2	46·3	53·6	16·3	25·0	38·9
Totals	100	100	100	99·9	99·9	100
Number of individuals of each class examined	125	160	28	172	304	36

TABLE 5.—Comparison of the degrees of enlargement of the posterior cervical glands among the children of Ikot Offiong and Mpok in the Eket district of Nigeria with those of the Gambia and the Congo.

AGE	MALES, 0—13				FEMALES, 0—11			
Place	Ikot Offiong	Mpok	Gambia, 1911	Congo, 1903-05	Ikot Offiong	Mpok	Gambia, 1911	Congo, 1903-05
' + ' glands	27·6%	4·0%	0·4%	8·0%	17·5%	1·7	0·2%	6·2%
' + — '	48·3	39·2	2·2	26·8	52·5	20·3	1·7	23·7
' + — — '	17·2	53·6	42·0	22·9	20·0	61·6	28·0	22·4
' — '	6·9	3·2	55·4	42·3	10·0	16·3	70·1	47·7

children especially appeared to be more healthy. This fact, taken in conjunction with the prevalence of trypanosomiasis at Ikot Offiong and its rarity at Mpok, suggests that in the Eket district just as in the Congo and the Gambia there is a definite connexion between the occurrence of this disease and a high percentage of enlarged cervical glands.

In other parts of West Africa gland palpation has proved of the greatest assistance in the recognition of sleeping sickness. It is interesting, therefore, to compare the figures we have obtained in Eket with those recorded by the investigators in the Congo and the Gambia which have recently been summarized by Todd (1913). The condition in children is particularly significant (Table 5). With regard to considerable degrees (' + ' and ' + – ') of glandular enlargement, Todd writes 'There is certainly a close connection between these percentages of enlarged glands and the much more frequent occurrence of trypanosomiasis among natives of the Congo; it has already been made clear that, in the Congo as well as in the Gambia, enlarged cervical glands mean trypanosomiasis. In 1911, it was estimated that 0·8 % of the native population in the Gambia had trypanosomiasis; there, cases only occur sporadically. It is scarcely possible to estimate the probable percentage infected among the natives examined in the Congo in 1903-05, but it is certain that 50 %, and often more, of the natives in many of the villages visited were infected.' If enlargement of the cervical glands has a similar significance in Eket, and there seems little reason to suppose it has not, the incidence of trypanosomiasis must be great indeed, for within the bounds of a district of less than 700 square miles many thousands of cases must be congregated.

SYMPTOMS

The earliest record of sleeping sickness on the West Coast of Africa are unanimous in attributing a very heavy death rate to the disease (Atkins (1742), Winterbottom (1803), &c.). More recent investigations have proved, however, that in many parts the disease now occurs in a mild form. Dutton and Todd (1903) in the First Report of the Trypanosomiasis Expedition to Senegambia (1902) wrote 'Taking all our facts into consideration, we believe the disease, as it occurs in natives, to be a peculiarly mild one, and that it is at present impossible to recognise it clinically,' and Horn (1910) in reporting on sleeping sickness in the Volta River district of the Gold Coast records that 'Although sleeping sickness is endemic in this part of the Gold Coast, and has been known to the natives for many years past, yet it is not at present in epidemic form, neither does it appear to have produced any epidemic within the memory of living natives, nor does it form any profound part of their traditions.'

The earlier writers may very likely have recognized only the advanced cases, but there is some evidence that trypanosomiasis in the course of years becomes less deadly in the endemic centres, although it appears to be capable of regaining its virulence when imported into new districts.

Mr. J. H. Harris (1912) in a recent book writes of sleeping sickness 'this scourge would seem to be spending its force. Seven years ago Uganda recorded over 8,000 deaths from sleeping sickness within twelve months, and the latest Government report shows that there has been a gradual reduction until in the year 1910 there were only 1,546. Happily this encouraging feature is present on the West Coast also. The Congo suffered more than any other colony . . . . Now, however, the absence of the scourge in many of the old districts is quite noticeable. Villages that we knew to be swept by this plague ten years ago are once more flourishing, and in some cases where the birth rate was almost nil the villages are again joyous with the laughter of little children.'

In Nigeria also sleeping sickness occurs in a mild form, apparently most similar to that in the Gambia. The disease in Eket, however, presents certain peculiarities which are difficult to explain adequately by a reduction in the pathogenicity of the parasite, the weeding out of the more susceptible individuals, or the establishment of a partial immunity.

The death rate from sleeping sickness in Eket seems to be very small. Of the 222 cases identified up to the end of 1913, nine had proved fatal, giving a mortality of 4 % for this period. Several of these patients had died outside the camp, so that it is not certain that in every case death was due to trypanosomiasis. For this reason, and because it is probable that it is the worst cases that have hitherto come under observation, the figure given above is not likely to be too low a one. From other considerations also it might be expected that the mortality must be slight. The disease occurs especially amongst children, and if many of them died of it, one would expect to find a scarcity of adults, which is not the case. Eket is, indeed, one of the more densely populated districts of Nigeria.

The fact that the malady is of so mild a type does not necessarily minimize the gravity of the situation. Without further investigation it is not possible to forecast the results of its spread into neighbouring districts, where it might reassume the virulent form which, according to native tradition, it once had when it devastated whole towns. The position of Europeans and other non-natives must also be considered. Until the danger is proved not to exist, it would be wise for those responsible for the opening up of the country by roads and rail and for the recruiting of labour to remember the disastrous effects that have followed the introduction of sleeping sickness into other parts of Africa. It will be sufficient to recall the effect of removing the remnant of Emin Pasha's Sudanese soldiers into Uganda and Busoga to emphasize this point, and the notable instance recently given by Bernard (1913), who relates how sleeping sickness spread in all directions round Djema, where it had been first introduced with some labourers who had been brought to build a factory.

The symptoms exhibited by the patients in Eket were often of the slightest. A visitor to the isolation camp at Ikotobo, seeing the troops of children at play, or dancing on moonlight nights, would be struck by nothing so much as by the apparent good health, high spirits, and happiness of the inmates. Many of the patients indeed showed no signs of illness (Pl. XXII, fig. 16). Such symptoms as do occur are those common to trypanosomiasis all over Africa. It will therefore be unnecessary to describe them in detail, but the following brief notes will suffice to indicate the clinical aspect of the cases.

So many of the patients being children, it was impossible, in the majority of cases, to get a clear account of the onset of the disease. As a rule, glandular enlargement, of from one to five years' duration, was the only symptom complained of on admission. In addition, fever, headache, and pains in the head and neck, were generally mentioned. The majority of the patients had been recognised to be suffering from trypanosomiasis (*Insip Itong*) for more than a year.

The difficulty experienced in confirming the diagnosis of trypanosomiasis in some cases may be explained by the long history of the infection, as a successful reaction on the part of the organism to the trypanosome is accompanied by sclerosis and disinfection of the glands, and the disappearance of the parasites from the peripheral blood. The former process, according to Thiroux and D'Anfreville (1909), precedes the latter by some months. Our experience, that gland puncture often revealed the presence of parasites whereas blood examination never did, is not necessarily in disagreement with this view, since we have been unable to prove the absolute sterility of the blood by examination after centrifugalization or by inoculation into susceptible animals.

With but very few exceptions the physical condition of the patients was good. The growth and development of the children did not appear to have been retarded, and the adults were for the most part well developed and muscular. In a minority of the cases some degree of emaciation was present, but in only one or two was it at all marked (Pl. XXI, fig. 7).

In a majority of the patients, on the other hand, the facial expression was somewhat dull. This feature, which was seldom pronounced in the children, was in part due to oedema of the face, and especially of the eyelids, which was a common sign (Pl. XXII, figs. 11 and 15). A few of the adult patients presented the typical dull, heavy faces associated with sleeping sickness. Prominence of the eyeballs was noted in three or four cases (Pl. XXII, fig. 14). The symptom does not appear to be a general or an important one, and is only mentioned because Vix (1912) observed exophthalmos in sixteen out of seventy cases examined in German East Africa.

Some degree of fever was invariably present, the highest point being reached in the afternoon, and the morning temperatures being normal or sub-normal. The fever was irregular in degree, but was seldom excessive. In very few cases was any sign of periodicity observed, such as that described by Kérandel (1910). As, however, most of the children were suffering from a concurrent malarial infection (simple tertian, quartan, or most commonly malignant tertian), any such regular variation would probably have been masked or obliterated.

Disturbances of the alimentary system were almost entirely absent. The tongue was generally furred, but the appetite was unimpaired, and digestion appeared to be excellent. As usual in native subjects, an examination of the faeces revealed the presence of a rich and varied intestinal fauna. Ascaris, taenia, oxyuris and trichocephalus were all commonly found, and in addition, in a few cases, ankylostomes. In addition, trichomonas and a small spirochaete were frequently seen. The liver was slightly enlarged in about ten per cent. of the cases. Spasm of the pharynx was observed once. As in the case recorded by Thiroux and D'Anfreville de la Salle (1911) the condition was suggestive of rabies.

Enlargement of the lymphatic glands (Pl. XXII, fig. 12) was present in practically every case, and was not infrequently the only symptom observed. The natives themselves attached the greatest importance to this sign, and believed that by the complete excision of the glands the disease might be cured. As a rule, the condition was that of a polyadenitis. The glands varied greatly in size, and in the same individual both large soft glands and more or less indurated and fibrosed glands were often present. The glands in the parotid region were frequently involved, giving the patients a peculiar facial expression not unlike that associated with mumps (Pl. XXII, fig. 13).

As a rule the glands were painless, but in some cases they were said to be painful at certain times. Native belief associated the pain with the phases of the moon. When the moon was waning, it was said, the glands became tender and caused shooting pains to be felt in the neck and head. An adult infected with trypanosomiasis gave a very precise description of pain in the glands preceding their enlargement.

The spleen was almost always enlarged, a fact no doubt accounted for by the prevalence of malaria. Anaemia was also a common symptom, but was moderate in degree, the red cells seldom numbering less than 3,000,000 per c.mm.

The pulse was as a rule rapid, small, and of low tension.

Neither the respiratory system, nor the urinary system, appeared to be specifically affected.

Skin affections were generally present, and were of several types. Impetigo of the scalp was perhaps the commonest, but at the stage at which the patients came under observation was generally quiescent (Pl. XXI, fig. 10). The next most frequent condition was a papulo-pustular eruption distributed all over the trunk and limbs, but often most pronounced on the hands and knees. A scaly and very itchy erythema was also frequently seen, and a circinate urticaria was not uncommon.

Sexual symptóms, impotence and amenorrhoea, which are stated to be very early manifestations by Bernard, were met with in a few of the adult cases. In some instances menstrual disorders were the only complaints of the patients who came to the dispensary for treatment for these conditions, and were only discovered to be suffering from trypanosomiasis in the course of their examination. It is doubtful if impotence can be a common or pronounced symptom, for in every village visited children abounded, and, indeed, appeared to be unusually numerous.

Affections of the bones and joints were seldom observed, and those that were seen were probably rheumatic in origin. Rigidity of the flexor muscles of the left arm was present in one case. Cramps were never complained of.

Headache and neuralgic pains were commonly present. Serious involvement of the nervous system was, however, exceptional. Somnolence occurred in less than ten per cent. of the cases, and in very few indeed was there the tendency to fall asleep at inappropriate times (Pl. XXI, fig. 9). A lesser degree, characterised by listlessness and apathy, was more common. Speech was slow and deliberate in some of the patients who exhibited a tendency to somnolence. In one or two the gait was shuffling and uncertain. In one case a slight degree of unilateral facial paresis was present. The same patient, a boy of eight, had rigidity of the flexor muscles of the arm, and slight stiffness of the leg, on the same side of the body.

A fine tremor of the tongue was common. Coarse tremors of the limbs and tongue were present in advanced cases. The deep reflexes were usually somewhat active. Romberg's symptom was sometimes noted. Deep hyperaesthesia was also observed occasionally. In one case epileptiform fits occurred. They came on suddenly one evening, and continued until about 2 a.m. the next morning. The next day the patient, although still somewhat dazed and stupid, seemed to have resumed her usual condition.

In the majority of cases intelligence appeared to be unimpaired, and several of the children were even so unusually acute as to be almost precocious.

TREATMENT

The routine treatment adopted at the camp has been a weekly intramuscular injection of 6 grs. of atoxyl. The patients appear to tolerate this dose, and up to the present no ill results have followed.

It would be difficult to judge of the action of any drug on a trypanosomiasis of the type found in Eket, for not only are the parasites undiscoverable in the blood and very rare in the gland juice, but also spontaneous cures frequently take place. The effects of treatment might, however, be estimated by the action on the temperature, and on the phenomenon of auto-agglutination of the red blood corpuscles.

As yet it is too early to give any definite opinion on the effects of atoxyl treatment at Ikotobo. In a few cases admitted to the camp in a greatly debilitated state the beneficial action has been striking, and the majority appear to have improved under treatment. Reduction in the size of the cervical glands, the sign that would appeal most strongly to the patients themselves and to their parents, has not yet been conspicuous. For the reasons given above it is impossible at present to determine to what extent the beneficial action of atoxyl has been due in its effect on the trypanosomes, and to what extent to its tonic properties.

Intercurrent infections, as, for instance, malaria, have of course been treated also at the camp. The general improvement in health attained in this manner may account in part for the good results observed.

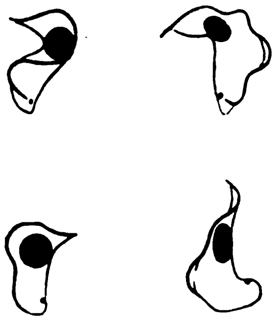
THE PATHOGENIC AGENT

In his 'General Report for the year 1912,' Dr. Foran wrote with regard to the parasite he had found in gland juice from the cases of sleeping sickness in Eket: 'Judging from measurements and general characteristics, I believe this parasite to be the well-known Trypanosoma Gambiense.' In a former paper, however, one of us (Macfie, 1913) described the morphology of the trypanosome as it appeared in the blood of a guinea-pig that had been infected by inoculation with cerebro-spinal fluid from one of the cases under observation at Ikotobo, and as it presented certain peculiarities, proposed for it the name *T. nigeriense*. The virulence of the parasite to the smaller laboratory animals has been found to be extremely low, a fact which has been considered to support the contention that it is not the same species as *T. gambiense*.

During the present investigations, further evidence has been sought on the nature of the pathogenic agent. As already pointed out, the parasites were only found in the gland juice, and in very small numbers. The forms encountered were similar to those previously described, and in one instance a very small individual of the type believed by us to be characteristic of *T. nigeriense* was found.

Guinea-pigs and white rats were the only animals available for experimental purposes. White rats had already been proved to be refractory, and it was not surprising that all attempts (4) to infect them proved unsuccessful. Three out of twelve guinea-pigs were infected by inoculation with blood containing trypanosomes of the strain obtained from Eket earlier in 1913, and it was anticipated that it would be possible to infect this species of animal from other cases of sleeping sickness. Seven guinea-pigs were inoculated with gland juice known to contain trypanosomes from as many cases of human trypanosomiasis, but only one became infected. A guinea-pig was also injected intraperitoneally with 5 c.cm. of blood from a proved case of sleeping sickness, but failed to become infected. A single wild mouse (*Mus musculus*) was inoculated, with a negative result.

The single guinea-pig that became infected had been inoculated subcutaneously with gland juice from one of the cases of sleeping sickness admitted to the camp in December, an adult male. The animal had been under close observation for seven weeks before inoculation, and had been kept in a double-fronted mosquito-proof cage. The trypanosomes first appeared in the blood in sufficient numbers to be detected on the twenty-seventh day. For several days (4) before this their presence had, however, been suspected, owing to the occurrence of well-marked auto-agglutination of the red cells. The parasites were at first extremely rare. On each of the first five days of the infection ten trypanosomes were found with difficulty, and were measured (Table 6). The longest of these fifty individuals measured 24 μ, the shortest 13 μ, and the average length was 17·54 μ. In the course of a day or two, however, the trypanosomes became rather less rare, although still far from numerous, and both longer and shorter forms were found. Some of the latter were of the type which we believe to be characteristic of this strain (see Text-figs).



× 2000. *T. nigeriense*, small forms characteristic of the strain, for comparison and contrast with the atypical stumpy forms of other polymorphic trypanosomes occasionally found in guinea-pigs (see Pl. XXIII, figs. 4, 5 and 6).

The extreme rarity or complete absence of trypanosomes from the peripheral blood of all the human cases examined, the rarity of the parasites even in the gland juice, and the difficulty experienced in infecting animals by inoculation, suggest that it must be a very exceptional occurrence for a tsetse fly to become infected by feeding on these cases. It has been estimated by one of us (1912) that the average amount of blood taken up by *G. palpalis* in an uninterrupted feed is about 3 c.mm. If, therefore, one of these flies fed fully on an infected human being every day, it would be over four years before it had ingested 5 c.cm. of blood, an amount which, in the experiment referred to above, failed to infect a guinea-pig when inoculated into its peritoneal cavity!

It is possible, of course, that at some stage of the disease trypanosomes may be present in the blood in considerable numbers. It is also possible that the blood may contain elements capable of infecting tsetse flies, but incapable of infecting vertebrates. Both these hypotheses are unsupported by any evidence, and are at least improbable. It seems, therefore, that the human infections must be dependent on some other cycle of development, including an insect and some so far unidentified animal host, the reservoir ot the disease. The ordinary development of the trypanosome may take place in these two hosts. The insect, infected from the animal host may, however, be capable of infecting human beings; but the disease may be so modified in them that they are incapable of handing on the infection any further. In this connexion the results obtained by Bruce and his collaborators (1913, 1) in the case of *T. simiae* are of interest. They found that monkeys were readily infected with this trypanosome by the bites of *G. morsitans*, but that inoculations with blood of goats containing the same

TABLE 6.—Measurements of the human trypanosome during the first five days of an infection in a guinea-pig.

Day of the infection	Number measured	LENGTH IN MICRONS		
		Average	Minimum	Maximum
1	10.	18·9	16	24
2	10	17·8	13	24
3	10	17·1	14	21
4	10	17·5	14	22
5	10	16·4	14	19
	50	17·54	13	24

parasites were usually unsuccessful, the passage of the trypanosome through the body of the goats having reduced its virulence for monkeys almost to nothing.

A similar reduction of the virulence may take place in the case of the human trypanosome in its passage through the bodies of the natives of Eket. The question then arises whether the unusual symptomology, and the atypical morphology and pathogenicity of the parasite, may not be accounted for by the modifying influence of the human host, and whether it is necessary to suppose that *T. nigeriense* and *T. gambiense* are specifically different. It is recognized nowadays that, in the words of Bruce and his colleagues (1913, 2): 'It is absurd to expect to arrive at any classification at all approaching a true one by the study of strains of trypanosomes kept for many years and undergoing many vicissitudes in our European laboratories,' but although the pathogenicity may undoubtedly vary, we think it has yet to be proved that the morphology may undergo definite changes, except as the result of the action of certain drugs. Such, at any rate, appears to be the significance of the experiments of Blacklock and Yorke (1913) with *T. vivax*. These investigators succeeded in modifying the virulence of this parasite so that it became pathogenic to rabbits, but after passage through six generations of these animals they found that 'the trypanosomes appeared to be identical with those seen in goat's blood.' While admitting the possibility, which in the interests of simplicity would be very readily entertained by us, we find ourselves unable at present to convince ourselves that the strain of human trypanosome found in Eket is identical with *T. gambiense*.

THE INVERTEBRATE HOST

The rarity of *G. palpalis* in the parts of the Eket district especially affected by sleeping sickness has already been mentioned. It is difficult to understand how so small a number of these insects can be responsible for so large a number of cases, especially when the absence of the parasites from the peripheral blood of the human patients is taken into consideration. On the eastern side of the district, *G. palpalis* is certainly more common, but in this part of the country sleeping sickness is much less frequent. It is impossible, however, to exclude *G. palpalis* as a factor in the transmission of the trypanosomiasis, for in every place which has been carefully and systematically searched it has been found, although, perhaps, with extreme rarity.

G. tachinoides, on the other hand, is much more abundant, and is particularly associated with the parts of the district in which sleeping sickness is most prevalent. A number of experiments were carried out to determine with what trypanosomes these flies were naturally infected. A full account of them is given below (Appendix 2), but it may be stated here that wild flies were found to be capable of transmitting *T. pecaudi* (*T. brucei*, of Uganda), *T. vivax*, and *T. pecorum*. Owing to the relative insusceptibility of the experimental animals employed to the Nigerian strain of human trypanosome, these experiments cannot be regarded as excluding the possibility that the wild *G. tachinoides* may also be infected with this parasite. Indeed, since they are capable of transmitting the three trypanosomes recorded above, it seems not improbable that they may also transmit *T. nigeriense*. Assuming that the disease is transmitted by a tsetse fly, it seems more probable that it will prove to be by *G. tachinoides* than *G. palpalis*.

It is now well known that several pathogenic trypanosomes, including *T. gambiense*, can be transmitted by more than one species of tsetse fly; and the conclusion to be drawn from the work that has been done on this subject would seem to be that they may develop in any species of *Glossina*. It still remains uncertain whether particular tsetse flies are specially adapted for the development of certain trypanosomes. In the case of *T. gambiense* development has generally been supposed to take place in *G. palpalis*, although Kleine and Fischer have found it occur in *G. morsitans* also. It seems reasonable to suppose, therefore, that *G. tachinoides* may also act as the invertebrate host. Whether such an association would be attended by any modification of the virulence of the parasite, or by such morphological changes as those described by us in the case of *T. nigeriense*, is at present unknown.

The members of the French Commission, impressed by the absence of parallelism between the frequency of occurrence of *G. palpalis* and that of cases of sleeping sickness, and having encountered many instances in which several members of a family living in the same hut were infected while other natives of the same village were not attacked, concluded that the agent of infection must be an insect biting in the night. *G. palpalis*, they stated, was the principal agent of infection. Its presence was absolutely necessary for the propagation of the disease to a distance, and for the maintenance of endemicity; but in addition, they thought, mosquitos and probably other biting insects might be important auxiliaries.

A similar lack of parallelism between tsetse flies and sleeping sickness seems to exist in Eket. In several instances also two or three members of a family were found to be infected, and the natives appear to believe that the disease may run in families. For these reasons it was thought possible that some other insect might play a part in the dissemination of the disease.

It has already been mentioned that impetigo of the scalp was a frequent and early symptom in our cases of trypanosomiasis. In several instances this condition of the skin was clearly stated to have preceded the onset of the disease. Why this impetigo should occur at all it is difficult to understand, as it is not, we believe, a common disease of native children. On one occasion it was suggested that it had originated in the bites of some insects, and as bugs (*Cimex lectularius*) are very common parasites of the natives, it was considered of interest to examine these insects with a view to determining if they were capable of transmitting trypanosomiasis.

Twenty-three bugs, collected from the beds of sleeping sickness patients, were therefore fed on a guinea-pig for eighteen days. At the end of this period only two of the bugs remained alive. The guinea-pig was kept under observation for forty-three days, but trypanosomes were never found in its blood, neither was auto-agglutination of the red cells noted. But little reliance can be placed on the result of this single experiment, especially as guinea-pigs are not highly susceptible to the human trypanosome.

THE ALTERNATIVE VERTEBRATE HOST OR RESERVOIR

Unfortunately it was impossible during the short time at our disposal to determine the reservoir of sleeping sickness in Eket. Neither the necessary material, nor the requisite experimental animals, for the purpose could be obtained, and the subject will have to remain for some future investigation.

With regard to game, the country is too well populated to harbour much, and, in fact, it is rarely met with in the greater part of the district; and what there is, unless it is assumed that the great majority is infected, seems insufficient to account for the number of human beings found to be suffering from trypanosomiasis. We were unable to procure a single antelope for examination, although we offered to the natives a substantial reward for every animal they would bring into the camp.

In the few examinations it was possible to make we failed to implicate domestic animals. The natives of Eket are still somewhat shy of Europeans, and were reluctant to allow their domestic animals to be examined. In almost every village, sheep, goats, dogs, dwarf cattle, and occasionally pigs were found. Trypanosomiasis was found to occur in these animals, and the dwarf cattle were infected with a minute babesia, probably *Theileria parva*, which, however, did not appear to cause any symptoms of illness. It would be interesting to know if this infection is in any way connected with the apparent immunity of these cattle to trypanosomiasis.

Altogether sixteen animals were examined, namely, four sheep, four goats, two dwarf cattle, four dogs, and two pigs; and of these four were found to harbour trypanosomes. *T. vivax* was found in one sheep and one dwarf cow, and a trypanosome of the type of *T. pecorum* in one pig and one sheep. All four animals appeared to be healthy. From the feeding experiments with wild *G. tachinoides* it is evident that *T. pecaudi* (*T. brucei*, of Uganda), *T. vivax*, and *T. pecorum*, at any rate, must be common in the district, either in the domestic or wild animals, or in both. No domestic animal was found to harbour trypanosomes of the human type.

In a recent discussion on the relation of big game to sleeping sickness, it was suggested that there might be human 'carriers' of trypanosomiasis. Prof. Yorke (1913), who opened the discussion, stated that in the case of *T. rhodesiense* 'The hypothesis that human beings can harbour the parasites for long periods without detriment to health, and thus act as reservoirs of the virus in the same way as the game,' was one which he could not support. In the case of a parasite with but a slight degree of pathogenicity, however, the possibility of there being human 'reservoirs' is greater, a view apparently held by Dutton and Todd (1903) when they wrote of *T. gambiense* in Senegambia: 'The slight virulence of this trypanosome, and the consequent lack of symptoms, has frequently suggested to us the possibility that the natives in this disease may bear the same relation to the Europeans as does the wild game of Central Africa to domestic animals in the tsetse fly disease.'

Bearing in mind the number of infected persons and the chronicity of the disease in Eket, it might be thought that in this district human beings might act as the reservoir. The fact that trypanosomes have not been found in the peripheral blood in a single case is undoubtedly a strong argument against this hypothesis. But as we have not been able to centrifugalize blood it must be remembered that the parasites may actually be present, but in such small numbers as to escape detection in an ordinary examination.

SUMMARY AND CONCLUSIONS

There is in the Eket district of Nigeria an endemic focus of sleeping sickness of considerable magnitude, and it is probable that a large percentage of the poulation either is or has been infected with the disease.

The disease is of a mild type, the mortality is low, and spontaneous cures appear to be frequent. The patients are mostly children.

Although trypanosomes can be found in the gland juice, they have never yet been detected in the peripheral blood. These trypanosomes differ in some respects in their morphology and their pathogenicity from typical strains of *T. gambiense*, and we believe that the two parasites are not of the same species.

The species of insect that transmits the disease in Eket to man, and the animal that acts as the reservoir of the virus, have not yet been determined. There is some reason to suspect that *Glossina tachinoides* may be found to carry the infection.

APPENDIX I

NOTES AND OBSERVATIONS ON SLEEPING SICKNESS IN THE EKET DISTRICT

BY

W. C. W. EAKIN

History. In the beginning of 1906 a native of Ikotobo came to the Mission Dispensary asking for medicine for sleeping. He mentioned that the disease was known to the natives as *Odono Idap*, or Sleeping Sickness. The writer thought it advisable to go and see the patient, and found a young adult woman lying under the veranda of a native hut, apparently dying. The husband stated she had been ill for over a year with a sleeping sickness, and was incapable of taking care of herself, as on one occasion she fell asleep while cooking food, fell into the fire, and was badly burned. The woman lingered for a short time and died a month later.

The suspicious nature of this case with its attendant symptoms raised the interesting question as to whether the sleeping sickness known in other parts of Africa had made its way to Southern Nigeria.

Again, in May, 1911, a chief from the town of Ikot Offiong, in the Oron part of the district, brought his daughter, a girl of about sixteen years of age, for medicine. The father stated she suffered from pains in the head followed by sleeping, and was unable to do her work. The girl appeared very ill, and the glands of her neck were abnormally enlarged. She slept whilst the chief described her case. He pointed to the glands, and asked if they could be cut out, as he believed their excision would effect a cure. He also mentioned that his eldest daughter had died the previous year with the same disease. Nothing could be done for her, and it was reported that she died a month later.

From recent information it appears the father of the girl also died of sleeping sickness, making a death roll in that family of three persons inside the last three years.

It was thought that these facts should be reported without delay, and Mr. S. A. Bill, of the Qua Iboe Mission, from information supplied by the writer, forwarded them to the Medical Officer, Calabar. Action was taken in due course, and Dr. R. W. Gray visited the district to investigate the matter. He established the diagnosis of sleeping sickness by finding the trypanosomes. He was followed by the late Dr. P. F. Foran to continue the investigation, and adopt measures to check the spread of the disease. At his request the following notes were obtained from chiefs and others resident in the district, and they may be taken to represent the native point of view, without modification by the writer, as to the history of sleeping sickness, its origin, infectious nature, and the measures adopted to prevent it spreading, and it is hoped they may throw some light on the matter.

Prevalence. Sleeping sickness is prevalent throughout the district with the possible exception of Ibuno—where a small tribe of Ibunos has settled at the mouth of the Qua Iboe River—and the natives assert that it is widely distributed.

Names. Two stages are known and recognised. The first characterised by gland enlargement called *Insip Itong*, and the second or somnolent stage as *Odono Idáp* (pronounced Awdongaw Edap).

Tradition. While too much credence cannot be given to statements made by natives, yet the majority of those interviewed affirmed that:—It is generally believed that the town of Ikotobo, very many years ago, was nearly depopulated by an epidemic of sleeping sickness. They state the disease is less fatal now owing to successful treatment by the native doctors in removing the enlarged glands. A strong feeling has grown up among chiefs and old people that it is now greatly on the increase, and especially in the towns and villages situated on the banks of the Obium River. The reasons for holding this opinion are that:—

1st. The laws controlling the habits of those suffering from *Insip Itong* are now disregarded.

2nd. Isolation is no longer considered necessary.

Cause. Information has been sought for from many sources as to the cause of sleeping sickness. The natives believe it can be contracted in any of the following ways:—

1st. By the shooting of a flaming or burning arrow from the gun or bow of a witch doctor.

2nd. By coming in contact with an infected person, such as eating with his spoon, wearing his cloth, or sleeping on his bed.

3rd. By a strange native laying his hands on the head of any person and invoking the evil spirits to cause the sickness.

In addition to the reasons given above, an exception was found in Ikotobo, where some natives attributed sleeping sickness to the bite of a fly known as the *Nsung Idap*, or sleeping fly. This insect is commonly known to every town in the district as the *Nsung Obio* or town fly. This fly has been identified as a tsetse fly.

Laws and Customs. An ancient code of laws and customs is in existence for controlling the habits of those suffering from *Insip Itong* and sleeping sickness. The general belief is that both stages are infectious, and that isolation is necessary to prevent it spreading.

The code is composed as follows:—

(*a*) Persons suffering from *Insip Itong* must provide their own spoon, plate, and cooking pot.

(*b*) They are forbidden to:—
  1st. Wash in the public watering-places.
  2nd. Use the cloth of another person.
  3rd. Sleep on a bed or use a mat of another.
  4th. Eat rice or any food of a granulous nature.

(*c*) If infected persons visit a native house they must not sit on a mat or seat, nor eat with a spoon or use any utensil. They must sit or sleep on the ground, eat with their fingers, using plantain leaves for plates.

(*d*) All persons in the *Insip Itong* stage are considered unclean until the glands are removed by a native doctor.

(*e*) When the glands are removed notice is given to the chiefs, and a day is appointed for the ceremony of receiving back to society persons pronounced cured by the native doctor. The whole town is called to the public meeting-place where it is to take place. The ceremony begins by presenting the cured persons to the chiefs. This is performed by the native doctor. The clothes they wore and the beds, with the utensils they used, are at this stage brought and burned publicly. A sacrifice of a fowl is made to the spirits by the doctor, who next proceeds to tie round the neck of his late patients a charm consisting of a shell enclosing a palm kernel. This is worn as a sign that the disease has been cured. A feast is then prepared in their honour, and the day ends up with dancing and singing.

Particulars. It is stated that the disease is equally distributed over males and females, and that the majority of those infected are children and young adults. If adults contract it the prevailing belief is that they cannot be cured.

Occupation. Natives of the trading, but more particularly of the farming, class, suffer most from sleeping sickness.

The belief is held that it can run in families, and cases have been cited where the father died, followed by his son and other members of his house.

It cannot be stated definitely that any particular place is associated with the disease, but particular towns and villages are known by the natives to suffer more than others.

Symptoms and Course. The first symptoms and the order in which they come in the case of children are given as follows:—

1st. Gland enlargement.
2nd. Shooting pains in the head and neck.
3rd. Fever and night sweats.
4th. Rash covering the body.

If the glands are not removed a second stage is reached, when the patient becomes weak, subject to fits, somnolence, and death.

The course of the disease is more rapid in the case of adults, and the periods between the *Insip Itong* and sleeping stages may vary from six months to one or two years before death.

In children the first stage runs on for years before the second is reached. It is difficult to obtain reliable information from the natives as to the length of time from the appearance of the first symptoms till the second or sleeping stage was reached, and from that till death intervened. This is due to their inability to keep count for longer than one year. The case of the girl of sixteen mentioned in the beginning of these notes was brought to the Mission Dispensary in May, sleeping. She had reached the sleeping stage six months previously, and died the following month. No information was obtained as to how long she was in the *Insip Itong* stage.

While persons suffering from sleeping sickness become weak and emaciated, it has been noticed they are able to eat as well as when in health. Fertility is not diminished, but according to custom, a man having a wife suffering from *Insip Itong* must have the glands removed without delay.

Mortality is said to be greater among adults than children.

Treatment. *Insip Itong* doctors are found in many towns, and one town called Odoro Atabong has no less than seven. Their business is to excise the glands, from the neck, axilla, and groin. Their fees vary according to the wealth and position of the patient. It is almost impossible to calculate the exact amount they demand for their services, but it may be assumed that £5 is the lowest sum they are willing to take for the operation. The 'cure' may extend over two or three years, and consist of several operations. Their methods and surgical instruments are of a primitive nature. Guarantees must be given before they are willing to operate, and it is usual for a substantial instalment to be paid over when the first operation is complete.

A native doctor proceeds as follows:—

A small piece of bush having been cleared the patient is brought to the spot in the middle of the night by the doctor, accompanied by his parents or friends. A place is prepared for him to sit, and he is warned not to move. A fire is kindled and some burning faggots taken from it by the doctor and whirled round the head of the patient while some formula, invoking the spirit to make the sickness obedient to the knife of the operator, is used.

This ceremony finished, the patient is led home, his head shaved, and some root medicine rubbed into the scalp. This medicine has the effect of making the skin peel off.

The person is next tied hand and foot to the veranda post of the house and the operation begins. It consists of cutting out a number of glands from the neck and other parts of the body. Six or seven glands may be excised at the first operation.

It is stated that many persons recover, but it is not known how many die after the operation.

Diseases in Animals. There was a time when droves of pigs were kept in large numbers, but by an ancient law they were destroyed.

The natives believe that pigs are:—

1st. Unclean and bring sickness.
2nd. Increase the number of *Nsung Obio* or town fly.
3rd. Responsible for many skin diseases.

Inquiries have been made to find out if there was any connection between this law of prohibition and sleeping sickness, but without result.

These notes would be incomplete without making special reference to the work of the late Dr. P .F. Foran during the twelve months he spent in the district. He displayed marked ability, and the success he attained demands the highest praise. He was entrusted with the difficult tasks of obtaining information from native sources as to the history, cause, &c., of sleeping sickness, and of establishing an Isolation Camp for infected persons. These he undertook with unflagging energy and unwearying patience. Before he left he was rewarded with the confidence of the chiefs and people, and as a result of their co-operation infected persons began to pour into the Isolation Camp he had prepared for them. He was untiring in his efforts to make the camp a congenial place for the patients. A school was commenced, and knitting, basket making, &c., instituted, in order that the patients might be happy and contented in their new surroundings. No adequate tribute can be paid to the brilliant and hard work of this officer. He encountered initial difficulties from prejudice and opposition that would have defeated most men, but his keen attention to every detail for the welfare of his patients, coupled with his kindly disposition, won for him an esteem that will live long in the memories of those with whom he came in contact.

W. C. W. EAKIN.

IKOTOBO,
November 24th, 1913.

APPENDIX II

TRYPANOSOMES FOUND INFECTING WILD *GLOSSINA TACHINOIDES*

BY

J. W. SCOTT MACFIE, M.A., B.Sc.

Six experiments were carried out at Ikotobo in November and December, 1913, with the object of determining the species of trypanosomes transmitted, in nature, by *G. tachinoides*. In four cases the flies were fed on clean guinea-pigs; in one case on a clean rat; and in one case on a clean goat. All the animals were kept in double-fronted mosquito-proof cages to obviate the danger of accidental infections; and in order to exclude the possibility of mechanical transmission, the flies were starved for two days after capture before being given their first experimental feed. Unfortunately no monkeys were available for experiment, so that it was very unlikely that any evidence of infection with the human trypanosome could have been looked for considering the relative insusceptibility to this strain of the species of animal employed.

Altogether 145 flies were used. The details of the experiments are given below, and the general results are shown in the subjoined table (Table 7). Five of the six animals developed trypanosomiasis, so that at least five out of the 145 flies must have been infected.

TABLE 7.—Results of feeding wild *G. tachinoides* on uninfected animals at Ikotobo.

Experiment No.	Number of flies fed	Animal	Result
1	9	Guinea-pig	Negative
2	37	,,	*T. pecaudi* (*T. brucei*, Uganda)
3	25	,,	*T. pecaudi* (*T. brucei*, Uganda)
4	16	,,	*T. pecorum*
5	22	White Rat	*T. pecorum*
6	36	Goat	*T. vivax*

Three species of trypanosome were thus transmitted, namely, *T. pecaudi* (*T. brucei*, Uganda type) to two guinea-pigs, *T. pecorum* (*T. congolense*) to a guinea-pig and a white rat, and *T. vivax* to a goat.

Transmission experiments have been carried out by a large number of workers, but so far as I am aware *G. tachinoides* has not previously been proved to transmit in nature either *T. pecaudi* (*T. ugandae*), or *T. vivax*. The experiments described here, therefore, lend further support to the belief that pathogenic trypanosomes may, under suitable conditions, develop in several species of tsetse fly.

TABLE 8.—Proportions of wild *G. tachinoides* infected with trypanosomes.

Species of Trypanosome	Number of flies fed	Number of infections	Ratio of infected to uninfected flies
T. pecaudi (*T. brucei*)	145	2	1 : 72
T. pecorum	145	2	1 : 72
T. vivax	36	1	1 : 36

The percentage of *G. tachinoides* infected in the Eket district was extremely high. In the case of *T. pecaudi* and *T. pecorum*, to which all the three species of animal were susceptible, at least two out of the 145 flies must have been infected with each, giving a ratio of infected to not-infected flies of 1 to 72. In the case of *T. vivax*, to which guinea-pigs and rats are refractory, at least one out of thirty-six flies must have been infected. It is possible that, employing such a small number of flies, good fortune played a part, and that the ratios might not have worked out so high had a larger number of tsetse flies been available.

Although such a high percentage of the tsetse flies was found to be infected with a trypanosome characterized by posterior nuclear forms (*T. pecaudi*, *T. brucei* of Uganda), no human infection with this type of parasite (*T. rhodesiense*) has as yet been identified.

TABLE 9.—Animal reactions of the trypanosomes isolated from *G. tachinoides* at Ikotobo.

Strain of trypanosome	Source of the virus	Incubation period	Duration	Remarks
T. pecaudi (*T. ugandae*).—				
Guinea-pig No. 5	*G. tachinoides*	?	?	Died on the 32nd day after the first feed, and the 11th day after the last feed, of the flies.
Guinea-pig No. 9	*G. tachinoides*	7	33	
Rat No. 16	Guinea-pig No. 5	7	18	
Rat No. 23	Guinea-pig No. 9	5	19	
T. pecorum (*T. congolense*)—				
Guinea-pig No. 14	*G. tachinoides*	?	42	Died on the 42nd day after the first feed, and the 39th day after the last feed, of the flies.
Guinea-pig No. 29	Guinea-pig No. 14	9	47	
Rat No. 19	Guinea-pig No. 14	—	—	Did not become infected
Ram No. 28	Guinea-pig No. 14	7	57+	Alive and well on the 57th day
Guinea-pig No. 15*b*	Ram No 28	14	42+	Still alive on the 42nd day
Rat No. 7	*G. tachinoides*	?	107+	Flies fed on days 1-5. Alive and well on the 107th day
Rat No. 26	Rat No. 7	—	—	Did not become infected
Guinea-pig No. 15*a*	Rat No. 7	—	—	Did not become infected
Guinea-pig No. 372	Rat No. 7	—	—	Did not become infected
Dog No. 370	Rat No. 7	—	—	Did not become infected
Dog No. 371	Rat No. 7	—	—	Did not become infected
T. vivax—				
Goat No. 21	*G. tachinoides*	?	81+	Flies fed on the first 14 days. Alive and well on the 81st day, but still infected.
Guinea-pig	Goat No. 21	—	—	Did not become infected
Rat	Goat No. 21	—	—	Did not become infected

T. pecaudi (*T. brucei*) (Pl. XXIII, figs. 1 to 10).

In two experiments, infections with a polymorphic trypanosome resembling *T. pecaudi* or *T. brucei* (Uganda type, *T. ugandae*) were obtained by feeding wild *G. tachinoides* on guinea-pigs.

EXPERIMENT 2

In the first experiment the flies, after a preliminary period of starvation lasting two days, were fed on guinea-pig No. 5 as they were brought in by the collector. In this way altogether thirty-seven flies fed on the animal between November 21st and December 12th. On the latter date the guinea-pig was found to be infected, and the feeding of the flies was stopped. In this instance, therefore, it is not possible to state what was the incubation period.

The trypanosomes were fairly numerous at first, but became extremely scanty from the 4th to the 9th day after their first appearance in the blood. On the 10th day they were again fairly numerous, and on the 11th day (December 22nd) the guinea-pig died.

Morphologically the trypanosomes were somewhat unlike the forms of *T. pecaudi* (*T. ugandae*) usually seen in a guinea-pig. The stumpy individuals were smaller and rather more slender (Pl. XXIII, figs. 4-6), and forms with posterior nuclei were never found. Twenty trypanosomes were measured on each of the four days on which it was possible to find this number in the blood films taken (Table 10). The longest of these eighty individuals measured 35μ, the shortest 14μ, and the average length was 25·87μ.

A single white rat, No. 16, was inoculated from guinea-pig No. 5, and developed trypanosomes typical of *T. pecaudi* (*T. ugandae*). Posterior nuclear forms were abundant during the latter days of the infection, when the parasites were present in the blood in enormous numbers (Pl. XXIII, figs. 7-10). The incubation period in this rat was seven days, and death occurred on the 18th day.

EXPERIMENT 3

In the second experiment twenty-five starved *G. tachinoides* were fed once only, on December 13th, on guinea-pig No. 9. On the 7th day trypanosomes first appeared in the guinea-pig's blood, and on the 33rd day death occurred. A white rat inoculated with the blood of this animal on the 20th day became infected, the incubation period being five days, and the duration of the disease nineteen days. In both these animals the trypanosomes resembled typical specimens of *T. pecaudi* (*T. ugandae*) and posterior nuclear forms were found.

The trypanosomes isolated in these two experiments were definitely polymorphic, and morphologically identical with either *T. pecaudi* or *T. brucei* (*T. ugandae*). These two species of trypanosome would appear to be indistinguishable, and are probably the same organism. Posterior nuclear forms were found in both strains. Of 280 individuals measured the longest was 35μ, the shortest 14μ, and the average length was 24·78μ (see Table 10). The incubation period, and the duration of the disease, in guinea-pigs and white rats is compatible with the identification given above.

TABLE 10.—Measurements of *T. pecaudi* (*T. brucei* of Uganda).

Host	Day of the infection	Number measured	LENGTH IN MICRONS		
			Average	Minimum	Maximum
Guinea-pig 5	1	20	26·6	20	32
,,	2	20	26·0	17	34
,,	3	20	28·5	17	35
,,	10	20	22·4	14	32
Guinea-pig 9	4	20	28·75	24	34
,,	6	20	22·95	14	32
,,	8	20	21·1	17	29
,,	10	20	23·7	18	32
,,	12	20	27·85	22	32
Rat 16	2	20	24·7	20	28
,,	4	20	24·1	21	35
,,	6	20	22·25	18	32
,,	8	20	23·75	18	33
,,	10	20	24·4	18	32
		280	24·78	14	35

TABLE 11.—Distribution, by percentages, in respect of length of *T. pecaudi* (*T. brucei* of Uganda).

Host	Number measured	Distribution, by percentages, of lengths in microns										
		14	15	16	17	18	19	20	21	22	23	24
Guinea-pig 5	80	1	...	1	4	4	1	7	3	1	7	9
Guinea-pig 9	100	1	...	...	2	9	8	7	4	5	1	8
White Rat 16	100	...	...	...	...	3	2	6	14	12	16	12
Totals, to the nearest whole numbers ...		1	0	0	2	5	4	7	7	6	8	10

Host	Number measured	Distribution, by percentages, of lengths in microns										
		25	26	27	28	29	30	31	32	33	34	35
Guinea-pig 5	80	6	3	6	8	10	15	6	4	...	3	1
Guinea-pig 9	100	6	5	7	10	7	8	4	5	2	1	...
White Rat 16	100	11	9	4	4	1	...	1	2	2	...	1
Totals, to the nearest whole numbers ...		7	6	6	7	6	7	4	4	1	1	1

T. pecorum (*T. congolense*) (Pl. XXIII, figs. 15 to 31).

In one guinea-pig, and one white rat, on which wild *G. tachinoides* had been fed, infections were obtained with small trypanosomes of the *congolense-pecorum* type.

EXPERIMENT 4

In this experiment sixteen starved *G. tachinoides* were fed for three days, December 16-18, on guinea-pig No. 14. Up to the 23rd day no infection had been noted, and from the 24th to the 31st day it was impossible to make any examinations. On the 32nd day, however, a few trypanosomes were found in the guinea-pig's blood, and on the 42nd day the animal died. On the 38th day a ram, a guinea-pig, and a white rat were subinoculated from guinea-pig No. 14. The rat did not become infected. The ram showed trypanosomes for the first time on the 7th day, and the guinea-pig on the 9th day. In both animals the parasites were at first extremely rare, and might easily have been overlooked had not a prolonged and careful search been made for them.

The infection in the ram remained slight up to the 57th day, when the experiment was concluded. The trypanosomes had never been otherwise than scarce, and had on several occasions been absent from the blood films examined. The animal had never shown any symptoms of illness. A guinea-pig subinoculated with the blood of this ram first showed trypanosomes on the 14th day.

In the guinea-pig No. 29, on the other hand, the trypanosomes steadily increased in numbers as the disease advanced. On the 45th day the animal seemed to be somewhat unwell, and on the 47th day it died.

TABLE 12.—Measurements of *T. pecorum* (*T. congolense*).

Host	Day of the infection	Number measured	LENGTHS IN MICRONS		
			Average	Minimum	Maximum
Guinea-pig No. 14	... 1	25	13·36	11	16
,,	6	25	13·08	10	18
,,	8	25	13·92	10	18
,,	9	25	13·12	9	16
Ram No. 28 ...	... 16	20	15·20	12	18
,,	18	20	14·35	12	17
,,	20	20	14·50	10	18
,,	21	20	14·25	12	17
Guinea-pig No. 29	... 18	20	14·25	12	17
,,	19	20	12·75	10	16
,,	20	20	14·90	12	17
Rat No. 7 ...	... 1	10	15·9	13	21
,,	5	10	14·4	11	18
,,	6	10	15·7	12	20
,,	7	10	14·6	12	17
,,	26	20	14·7	11	18
		300	14·12	9	21

TABLE 13.—Distribution in respect of length of *T. pecorum* (*T. congolense*).

	Length in microns												
	9	10	11	12	13	14	15	16	17	18	19	20	21
Actual number of each length counted	1	6	17	32	67	52	52	37	25	7	2	1	1
Percentage, to the nearest whole number	...	2	6	11	22	17	17	12	8	2	1	...	...

Measurements were made of the trypanosomes in the blood of three out of the four animals infected with this strain (see Tables 12 and 13). In the original guinea-pig, No. 14, twenty-five trypanosomes were measured on the 1st, 6th, 8th, and 9th days of the infection. The average length of these hundred individuals was 13·37μ; the longest measuring 18μ, and the shortest 9μ. In the ram, and guinea-pig No. 29, it was impossible to find more than one or two parasites in the blood films taken on each of the earlier days of the infection, and so detailed measurements at this stage were out of the question. Twenty trypanosomes were, however, measured, in the case of the ram, on the 16th, 18th, 20th, and 21st days. The average length of these eighty individuals was 14·57μ; the longest measuring 18μ, and the shortest 10μ. In guinea-pig No. 29 twenty trypanosomes were measured on the 18th, 19th, and 20th days of the infection. The average length was 13·96μ, the maximum 17μ, and the minimum 10μ.

EXPERIMENT 5

Twenty-two starved *G. tachinoides* were fed in batches in this experiment on white rat No. 7 during a period of nine days. On the 15th day, that is the 7th day after the last day on which the flies were fed, trypanosomes appeared in the blood of the rat. The infection was a very slight one, and although trypanosomes were usually present in the blood, their numbers were always so small that it was impossible to find sufficient to make a satisfactory study of their measurements. On the 1st, 5th, 6th, and 7th days of the infection ten individuals were found with difficulty, and were measured (Table 12). The average length of these forty trypanosomes was 15·15μ, and the range was from 11μ to 21μ. An additional twenty were measured on the 26th day, and gave an average length of 14·7 μ, the longest measuring 18 μ, and the shortest 11 μ.

One rat, two guinea-pigs, and two dogs were subinoculated from this animal, but did not become infected. The rat itself was alive and apparently well on the 107th day when the experiment was ended. Latterly the trypanosomes had but seldom been found in the blood, and the animal appeared to be recovering from the infection. Phagocytosis of the red cells was observed in this rat.

Although this trypanosome (Pl. XXIII, figs. 24-31) differed somewhat in its morphology and animal reactions from the preceding one, isolated in Experiment 4 (Pl. XXIII, figs. 15-23), it has been included with it here, as there did not appear to be sufficient reason for differentiating it. The parasites were always very scanty, and the smaller forms, which in the other strain became more numerous as the infection increased, and which may be of pathological importance, were rarely seen or entirely absent. The difference between the average lengths of this trypanosome (15·00 μ) and that of the ram (14·57 μ) was, moreover, less than the difference between the strain isolated in Experiment 4 as it appeared in the original guinea-pig (13·37μ), and in the ram (14·57 μ) inoculated from it.

The trypanosomes isolated in these two experiments were of the *T. pecorum* or *T. congolense* type. They were short organisms without a free flagellum, although some forms had the anterior end drawn out to such a fine point as to simulate a free portion (Pl. XXIII, figs. 26 and 27). Morphologically they were identical also with *T. nanum*, a species which was supposed to differ from *T. pecorum* only in being uninfective to the smaller laboratory animals. Bruce (1913) has proved, however, that *T. pecorum* and *T. nanum* are really identical; and Blacklock and Yorke (1913) have recently brought forward evidence to show the identity of *T. congolense* and *T. nanum*. It seems probable that *T. pecorum*, *T. nanum*, and *T. congolense* are really all varieties of the same species.

T. vivax (Pl. XXIII, figs. 11 to 14).

Thirty-six wild *G. tachinoides* were fed daily on a young male goat from December 23rd, 1913, to January 5th, 1914. The goat had been under observation for several weeks, and its blood on repeated examination had always proved negative. The tsetse flies had all been starved for two days before being fed on the goat.

On January 5th, that is on the 14th day, a very few trypanosomes were found in the blood. On the following day the parasites were fairly numerous, and continued to be so until the 19th day. From the 20th to the 25th day they were very rare, but increased slightly in numbers from that date until the 31st day.

Coincident with the appearance of the trypanosomes the goat began to show symptoms of illness. He became unusually quiet, showed a tendency to lie down and sleep, and had a slight nasal discharge. The goat was still alive on the 81st day, and appeared to be in excellent health although still infected with the trypanosomes.

The trypanosomes showed all the morphological features characteristic of *T. vivax*. Twenty trypanosomes were measured on each day from the 2nd to the 6th day of the infection. On the first day, and for several days after the sixth day, it was impossible to find a sufficient number of parasites to measure. The average length of these 100 trypanosomes was 23·74 μ, the longest being 28 μ, and the shortest 19 μ (see Table 14). The percentage distribution in length is shown in Table 15.

Owing to the scarcity of experimental animals it was only possible to inoculate one white rat and one guinea-pig with this trypanosome. Neither became infected. From the morphology of the parasite, and these two experiments, there can be no doubt as to the identity of this trypanosome.

TABLE 14.—Measurements of *T. vivax*.

Day of the infection	Number measured	LENGTH IN MICRONS		
		Average	Minimum	Maximum
2	20	24·3	21	28
3	20	23·6	21	28
4	20	22·95	20	25
5	20	24·45	22	27
6	20	23·4	19	26
	100	23·74	19	28

TABLE 15.—Distribution, by percentages, in respect of length of *T. vivax.*

Length in microns

19	20	21	22	23	24	25	26	27	28
1	4	4	13	19	23	27	4	3	2

SUMMARY

T. pecaudi (*T. ugandae*), *T. pecorum* (*T. congolense*), and *T. vivax* are transmitted by *Glossina tachinoides* in the Eket district of Nigeria, and were isolated by feeding flies caught in the neighbourhood of Ikotobo on healthy animals.

REFERENCES

Bernard, — (1913). Relation d'une Tournee Médicale faite dans la Region du Djema et du M'Bomou (Oubangui-Chari) Avril et Mai, 1912. Ann. d'Hyg. et Méd. Colon, Vol. 16, No. 2, pp. 321-332.

Blacklock, B., and Yorke, Warrington (1913*a*). *Trypanosoma vivax* in Rabbits. Ann. Trop. Med. and Parasit., Vol. VII, No. 4, p. 563.

——— (1913*b*). The Probable Identity of *Trypanosoma congolense* (Broden) and *T. nanum* (Laveran). Ann. Trop. Med. and Parasit., Vol. VII, No. 4, p. 603.

Borchers, Carl (1912). Einige Beobachtungen über den Wundverlauf bei Schlafkranken. Arch. f. Schiffs u. Trop. Hyg., Vol. 16, No. 19, 648.

Bourret (1913). Recherches sur le Parasitisme Intestinal, la Dysenterie et la Maladie du Sommeil à Saint Louis Sénégal. Ann. d'Hyg. et Méd. Colon, Vol. 16, No. 2, pp. 283-307.

Bruce, D., Harvey, D., Hamerton, A. E., and Lady Bruce (1913, 1). Proc. Roy. Soc., Vol. B. 87, No. B. 592, pp. 48-57.

——— (1913, 2). Proc. Roy. Soc., Vol. B. 87, No. B. 592, pp.1-26.

Dutton, J. E., and Todd, J. L. (1903). First Report of the Trypanosomiasis Expedition to Senegambia, Liverpool, Memoir XI.

——— (1906). Liverpool School of Tropical Medicine, Memoir XVIII.

Greig, E. D. W., and Gray, A. C. H. (1904). Proc. of the Roy. Soc., LXXIII, p. 455.

Harris, John H. (1912). Dawn in Darkest Africa, pp. 132-133.

Hearsey, H. (1912). Sleeping Sickness Bulletin, Vol. IV, No. 37, p. 195

Horn, A. E. (1910). A Report on Sleeping Sickness in the Volta River District of the Gold Coast Colony, etc.

Kerandel, J. (1910). Un cas de Trypanosomiase chez un Médecin (Auto-observation). Bull. de la Soc. de Path. Exot., Novembre, p. 642.

Koch, R., Beck, M., und Kleine, F. (1909). Bericht über die Tätigkeit der zur Erforschung der Schlafkrankheit in Jahre 1906-07 nach Ostafrika entstandten Kommission. Arbeit. aus dem Kaiserl. Gesund. XXXI, Heft 1.

Macfie, J. W. Scott (1912). Experiments and observations upon *Glossina palpalis*. Bull. Ent. Res., Vol. III, pp. 69-70.

——— (1913). On the Morphology of the Trypanosome (*T. nigeriense*, n.sp.) from a case of Sleeping Sickness from Eket, Southern Nigeria. Ann. Trop. Med. and Parasit., Vol. VII, p. 339.

Martin, G., and Leboeuf, — (1908). Bull. de la Soc. de Path. Exot., Février, 1, p. 126.

Martin, G., Leboeuf, and Roubaud (1909). Rapport de la Mission d'Étude de la Maladie du Sommeil au Congo Français, 1906-1908.

Moiser, B. (1913). Notes on a few photographs illustrating the Haunts and Habits of *Glossina tachinoides* in Bornu, N. Nigeria. Bull. of Ent. Res., Vol. IV., pt. 2, p. 145.

Roubaud, E. (1911). Nouvelle contribution à l'étude biologique des glossines. Quelques données sur la biologie des *Gl. morsitans* et *tachinoides* du Soudan nigérien. Comptes Rendus de l'Académie des Sciences, No. 14, p. 637.

Sanderson, Meredith (1912). The Human Trypanosomiasis of Nyasaland. Trans. Soc. Trop. Med. and Hygiene, Vol. V, No. 8, p. 298.

SIMPSON, J. J. (1912). Entomological Research in British West Africa—III, Southern Nigeria. Bull. Ent. Res., Vol. III, pt. 2, p. 137.

THIROUX, A., et D'ANFREVILLE, L. (1909). De l'hypertrophie des ganglions dans la trypanosomiase humaine. Bull de la Soc. de Path. Exot., Juillet, p. 397.

THIROUX, A., et D'ANFREVILLE DE LA SALLE, L. (1911). La maladie du Sommeil et les Trypanosomiases Animales au Sénégal. Paris.

TODD, JOHN L., and WOLBACH, S. B. (1911). The Diagnosis and Distribution of Human Trypanosomiasis in the Colony and Protectorate of the Gambia. Ann. Trop. Med. and Parasit., Vol. V, No. 2, p. 245.

TODD, JOHN L. (1913). Concerning the Sex and Age of Africans suffering from Trypanosomiasis. Ann. Trop. Med. and Parasit., Vol. VII, No. 2, p. 309.

VIX, W. (1912). Psychiatrisch-neurologischer Beitrag zur Kenntnis der Schlafkrankheit, etc. Arch. f. Psychiatrie u. Nervenkrankheiten, Vol. 50, No. 1, p. 1.

WINTERBOTTOM, T. (1803). An account of Native Africans in the neighbourhood of Sierra Leone.

YORKE, W. (1911). Auto-agglutination of Red Blood Cells in Trypanosomiasis. Proc. of the Roy. Soc., B. 562, p. 238.

——— (1913). Journal of the African Society, pp. 29, 68.

EXPLANATION OF PLATES.

Plate XIX

Fig. 1. Ikot Offiong, a typical village of the Eket district.

Fig. 2. The Obium creek from the Qua Iboe river.

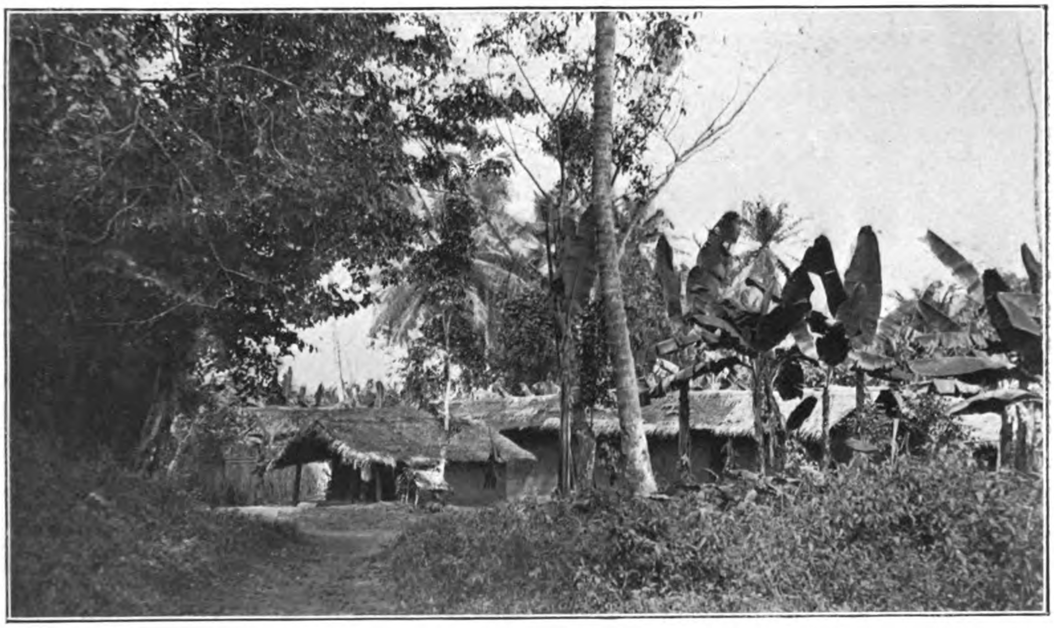


FIG. 1

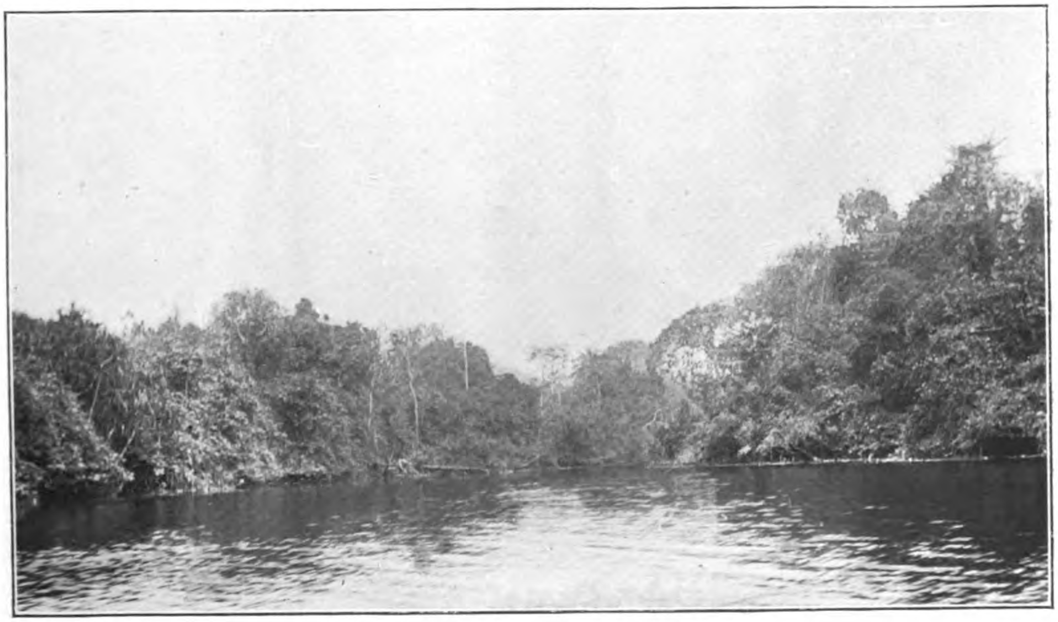


FIG. 2

C. Tinling & Co., Ltd., Imp.

PLATE XX

Fig. 3. The dispensary and hospital at the sleeping sickness camp, Ikotobo.

Fig. 4. The instruments used by the native 'doctors' for the excision of lymphatic glands.

Fig. 5. A sleeping sickness patient showing the scars left on the neck by the operation of excision of the glands as practised by the native 'doctors.'

Fig. 6. A few of the huts for the patients of the sleeping sickness camp, Ikotobo.

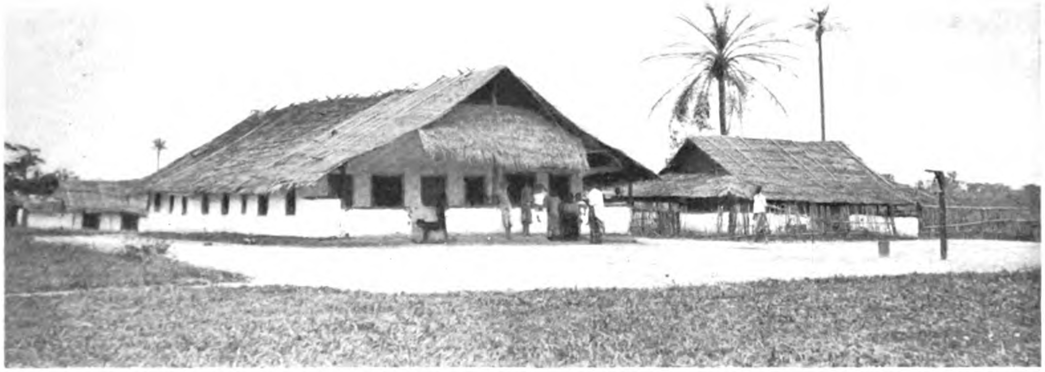


FIG. 3

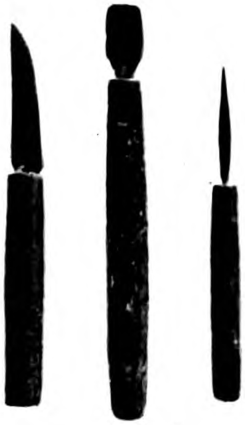


FIG. 4

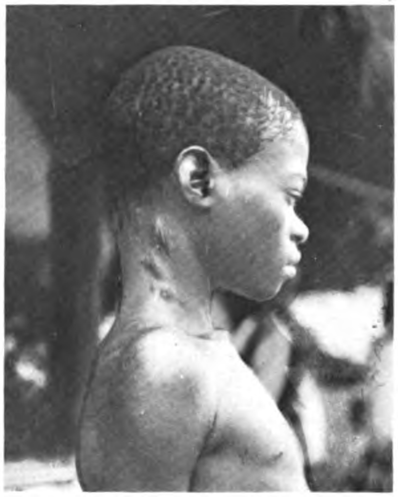


FIG. 5

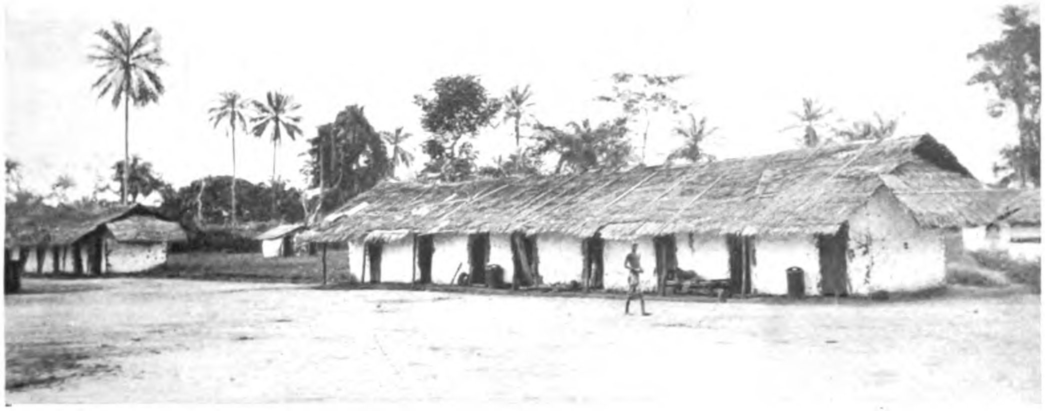


FIG. 6

PLATE XXI

Fig. 7. The oldest inmate of the camp.

Fig. 8. A little water-carrier, showing the absence of all protection against the attacks of biting insects.

Fig. 9. A case of sleeping sickness in the somnolent stage.

Fig. 10. A case showing the impetigo of the scalp.

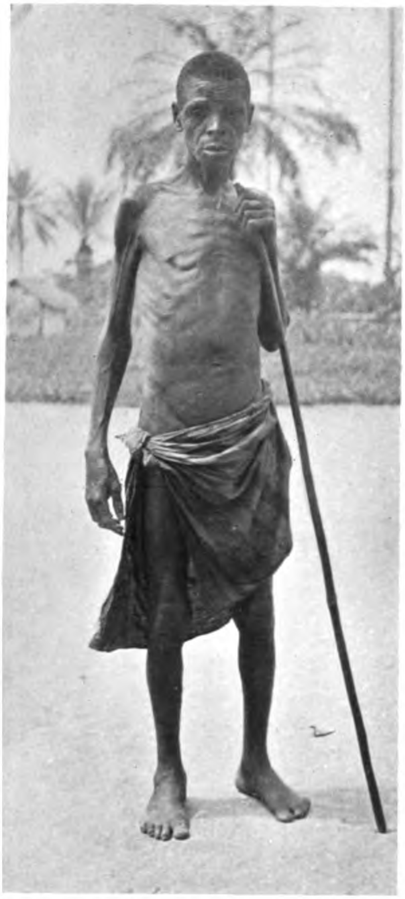


FIG. 7

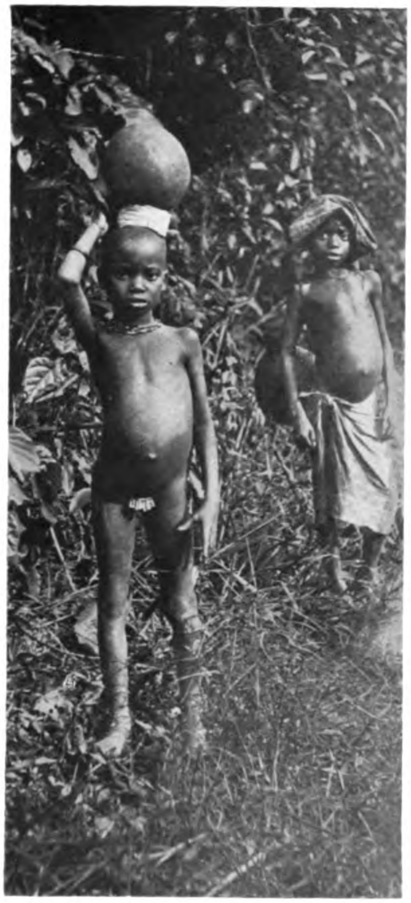


FIG. 8

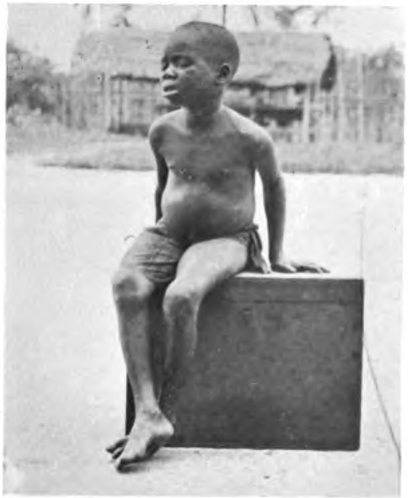


FIG. 9

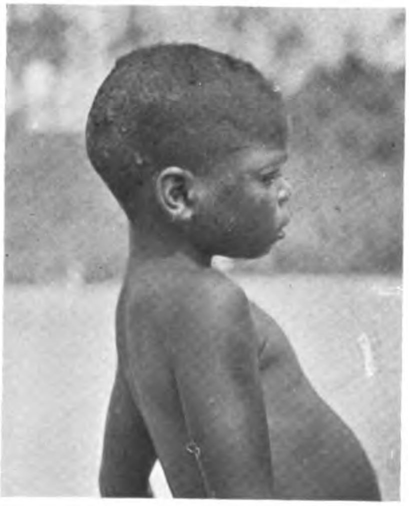


FIG. 10

C. Tinling & Co., Ltd., Imp.

PLATE XXII

Cases of sleeping sickness at Ikotobo showing:

Fig. 11. Facial oedema.

Fig. 12. Enlargement of the posterior cervical glands.

Fig. 13. Enlargement of the glands in the parotid regions giving the patient a mumps-like appearance.

Fig. 14. Slight prominence of the eyeballs.

Fig. 15. Oedema of the eyelids.

Fig. 16. The very healthy and happy appearance of many of the patients.

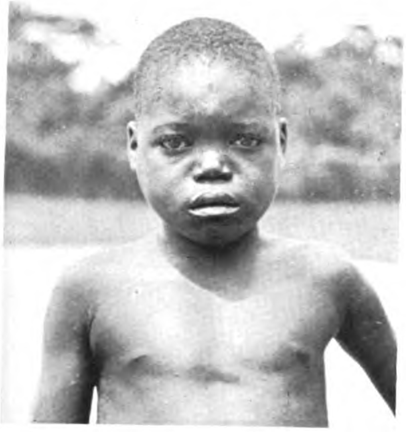


FIG. 11

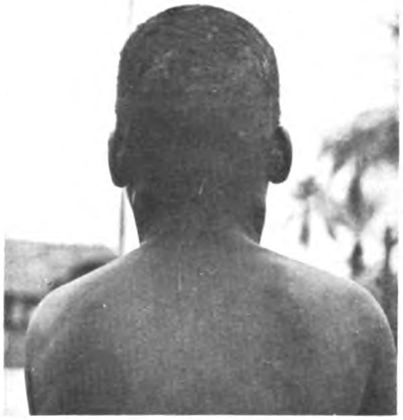


FIG. 12

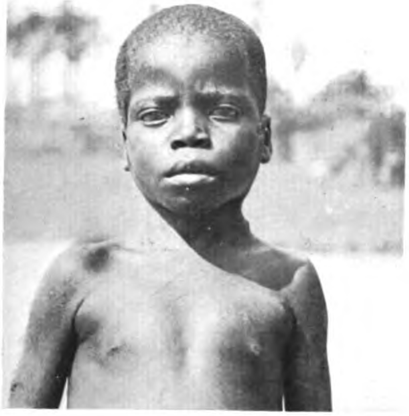


FIG. 13

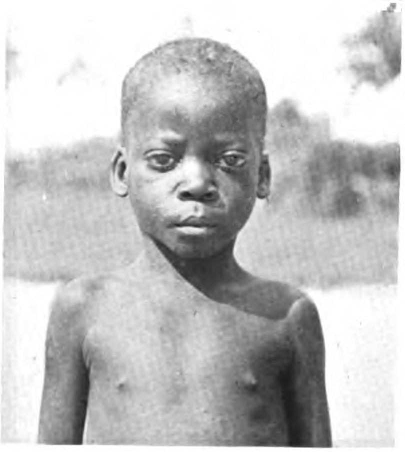


FIG. 14

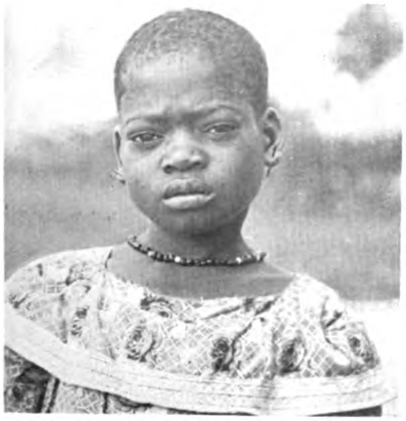


FIG. 15

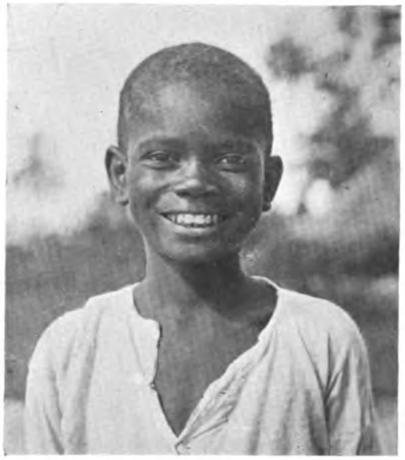


FIG. 16

C. Tinling & Co., Ltd., Im

PLATE XXIII

Trypanosomes isolated from wild *Glossina tachinoides*. × 2000.

Figs. 1-6. *T. pecaudi* (*T. brucei* of Uganda) from guinea-pig No. 5. Figs. 4, 5, and 6 illustrate the atypical stumpy forms found in this animal.

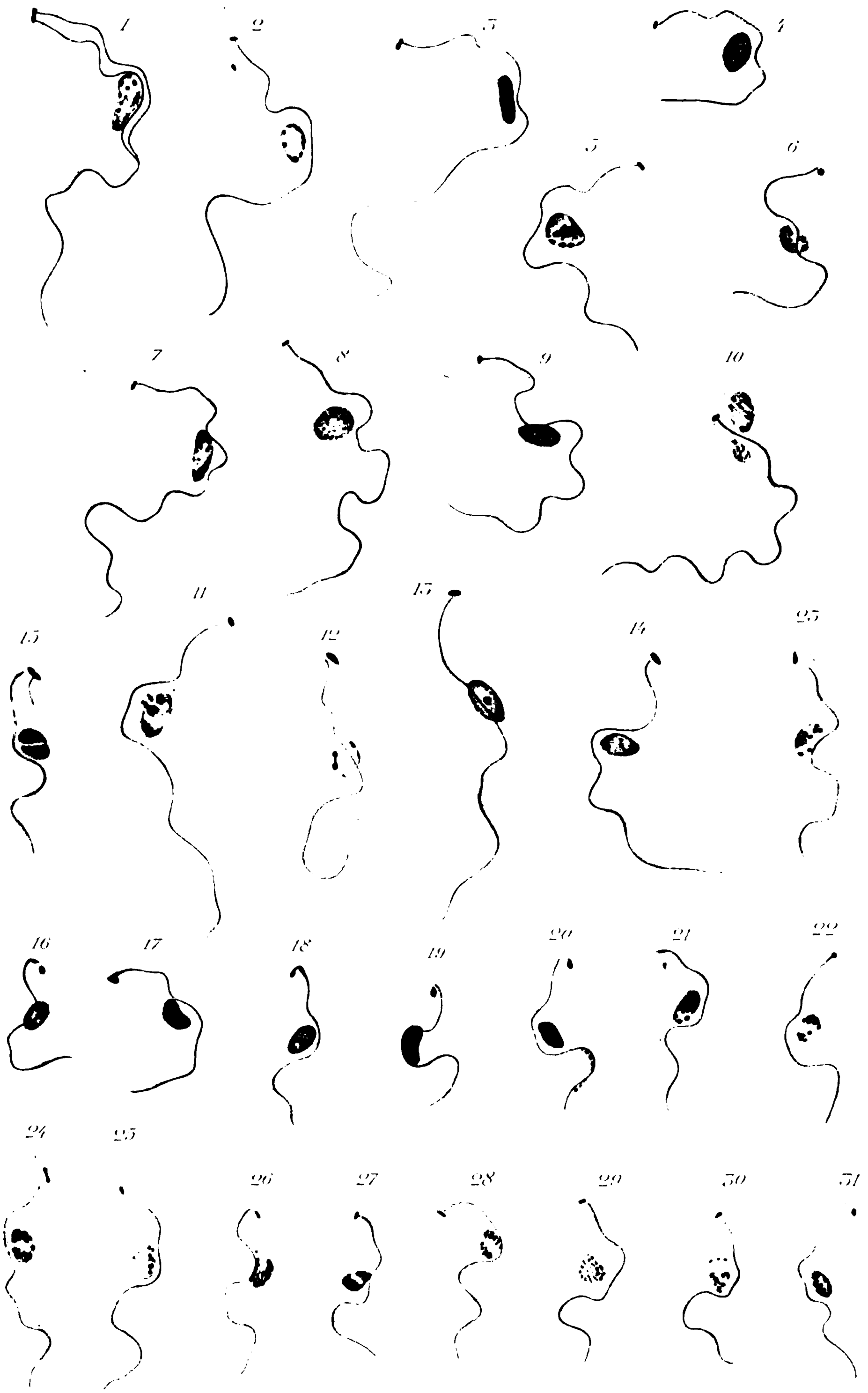
Figs. 7-10. *T. pecaudi* (*T. brucei* of Uganda) from rat No. 16. The same strain of trypanosome as figs. 1-6.

Figs. 11-14. *T. vivax* from goat No. 21.

Figs. 15-21. *T. pecorum* (*T. congolense*) from guinea-pig No. 14.

Figs. 22 and 23. *T. pecorum* (*T. congolense*) from ram No. 28. The same strain of trypanosome as figs. 15-21.

Figs. 24-31. *T. pecorum* (*T. congolense*) from rat No. 7.



M. Rhodes pinx.

TRYPANOSOME STRAINS ISOLATED FROM

WILD GLOSSINA TACHINOIDES

IST

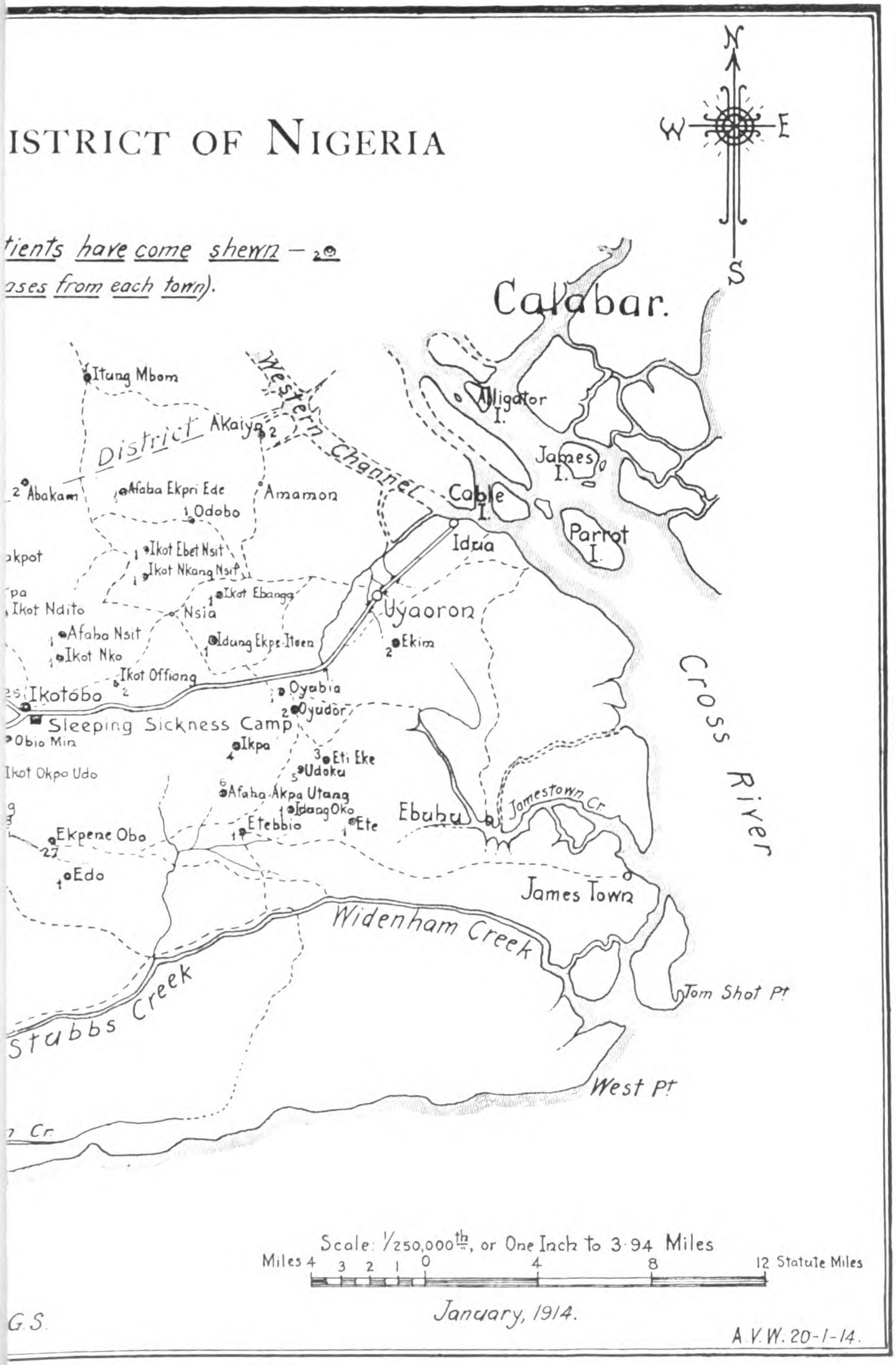
tients

ases

Sta

Cr

G.S



ISTRICT OF NIGERIA
tients have come shewn – 2●
ases from each town).
N
W
E
S
Calabar.
Itung Mbom
District
Akaiya 2
Western Channel
Alligator I.
James I.
Cable I.
Parrot I.
Idua
2 Abakam
Afaba Ekpri Ede
Amamon
1 Odobo
akpot
1 Ikot Ebet Nsit
1 Ikot Nkang Nsit
1 Ikot Ebanga
pa
1 Ikot Ndito
Nsia
Uyaoron
1 Afaba Nsit
1 Idung Ekpe Itoen
2 Ekim
1 Ikot Nko
6 Ikot Offiong 2
25 Ikotobo
Oyabia
2 Oyudor
Sleeping Sickness Camp
Obio Min
4 Ikpa
3 Eti Eke
5 Udoku
Ikot Okpo Udo
6 Afaha Akpa Utang
1 Idung Oko
9
3
Etebbio
1 Ete
Ebubu
Jamestown Cr.
Cross River
Ekpene Obo 27
1 Edo
James Town
Widenham Creek
Stubbs Creek
Tom Shot Pt
West Pt
Cr.
Scale: 1/250,000th, or One Inch to 3·94 Miles
Miles 4 3 2 1 0 4 8 12 Statute Miles
G.S.
January, 1914.
A.V.W. 20-1-14.

NOTES ON SOME BLOOD PARASITES COLLECTED IN NIGERIA

BY

J. W. SCOTT MACFIE, M.A., B.Sc.

MEDICAL RESEARCH INSTITUTE, LAGOS, SOUTHERN NIGERIA

(*Received for publication* 14 *April*, 1914)

PLATES XXIV, XXV

In the following pages a few notes on blood parasites collected in Nigeria are brought together, and with them is included for want of a better opportunity, the description of a spirochaete found in the gut of a tsetse fly. Individually they would, perhaps, have been too inconsiderable to record; and had it been possible further experiments and observations would have been made in every case.

The paper is arranged in the following sections:—

1. Preliminary note on the occurrence of babesiasis of domestic animals in Nigeria.
2. A spirochaete isolated from the blood of a guinea-pig.
3. Bacilliform bodies found in the red corpuscles of a rat.
4. A disease of fowls characterised by inclusions in the leucocytes.
5. Blood parasites of lizards and toads.
6. The occurrence of a spirochaete in the gut of *Glossina tachinoides*.

PRELIMINARY NOTE ON THE OCCURRENCE OF BABESIASIS OF DOMESTIC ANIMALS IN NIGERIA

Of the diseases of domestic animals in Nigeria considerable attention has been paid to trypanosomiasis, but so far but few observations have been made on babesiasis, and such as have been recorded have related mainly to the disease in dogs. No disease of cattle characterised by haemoglobinuria appears hitherto to have been reported, and for this reason perhaps the presence of babesiasis in these animals seems to have been entirely overlooked. The insignificant size of the parasites, moreover, which would easily escape detection unless especially searched for, may account for the scant attention paid to them.

In August, September, and October, 1913, blood films were obtained from the slaughter-houses at Calabar, Warri, and Onitsha, and a few were also collected at Lagos. Altogether fifty-nine animals were examined, fifteen goats, seventeen Hausa cattle, two dwarf cattle, and twenty-five sheep. In fifteen, two Hausa cattle, two dwarf cattle, and eleven sheep, babesias were detected; that is over 25 % of the animals were found to be infected. As some of the blood films were unsuitable for examination for minute parasites, it is possible that the percentage should be placed even higher than this.

TABLE I.—The results of 59 examinations of domestic animals in Nigeria for the occurrence of babesiasis.

Station	EXAMINED				Number infected with babesiasis
	Goats	Sheep	Hausa cattle	Dwarf cattle	
Calabar	12	...	17	...	2 Hausa cattle
Ikotobo	...	...	...	2	2 Dwarf cattle
Lagos	...	5	...	...	4 sheep
Onitsha	3	9	...	...	7 sheep
Warri	...	11	...	...	None
Totals ...	15	25	17	2	15

In the majority of the cases the parasites were numerous, but in three apparently healthy sheep examined at Lagos they were scanty. One of these sheep had been under close observation for two months before its blood was examined, and during this time had never shown any signs of illness, neither did it appear to be unwell at the time of examination, nor during the succeeding four months. Haemoglobinuria was never observed. The other two sheep were found living in a compound in Lagos in which a case of yellow fever had occurred. They appeared to be perfectly healthy animals, but on examination a few minute ring-shaped babesias were found in the red blood corpuscles (Pl. XXIV, figs. 1 and 2). The close resemblance of these parasites to the *Paraplasma flavigenum* of yellow fever is remarkable, but need not be discussed here.

The majority of the cases of babesiasis occurred in animals that were about to be slaughtered, and no evidence was available to show whether the disease was accompanied by severe symptoms or not. The infected animals examined at Lagos were healthy, but in them the parasites were rare. A somewhat larger infection in cows of the dwarf breed has recently been observed at Ikotobo. The animals were in splendid condition, and their urine was free from haemoglobin. In some of the sheep and cattle examined at Onitsha and Calabar considerable numbers of parasites were found. The animals in these cases had been imported into Southern Nigeria from the north. It is unlikely that such heavy infections could have been entirely benign.

So far as could be judged from the material available the babesias in all these animals, with two exceptions to be referred to later, were of the same species. The parasites were minute, seldom exceeding 1 μ to 1·5 μ in their longest diameters. They were pleomorphic. In stained preparations the most common forms were rounded, pyriform, or rod-shaped bodies. The chromatin as a rule consisted of a single minute dot at one end of the parasite, crescent-shaped, or horseshoe-shaped masses were, however, not infrequent, and occasionally two small dots were seen situated at the extremities of a diameter in the rounded and ovoid forms. In all the cases in which the parasites were numerous what appeared to be multiplication forms occurred, which consisted of groups of four chromatin grains arranged so as to form a cross.

The morphology of this parasite corresponds with the description given of *Theileria parva*. It should be mentioned, however, that Koch's blue bodies were never found, but as these are only occasionally seen in the peripheral circulation, this observation is not of much significance. Theiler, however, has described by the name *Piroplasma mutans* a minute parasite in cattle in South Africa and

Madagascar which resembles *Th. parva* but for the absence of Koch's blue bodies, and the innocuous nature of the infection. It is possible, therefore, that the parasites found in Nigeria may have belonged to this species, although they appeared on the whole to be smaller.

An attempt was made to cultivate this parasite after the manner of Bass. For this purpose blood was obtained from a ram by venipuncture, and transferred to a tube containing the glucose. The blood, after being defibrinated, was distributed into eight smaller tubes, and then incubated at 37° C. Inadvertently a rather stronger percentage of glucose was employed than that recommended by Bass; 1/10 c.cm. of a 50% solution being added to each 8 c.cm., instead of to each 10 c.cm. of blood.

In the blood of the ram, just before the experiment was started, parasites were fairly numerous. The great majority were simple forms, either rods, rings, or pear-shaped bodies, and dividing forms were rare, and did not exceed 4%.

After *six hours'* incubation the number of dividing forms had risen to 19%, and the simple forms appeared to have grown rather larger. Of the dividing forms 9% showed two chromatin dots, 2 % three, and 8% four. After *24 hours* the percentage of dividing forms was 21%, but no forms showing more than four chromatin dots were observed. The parasites continued to be well shaped and numerous up to *90 hours*, and the percentage of dividing forms remained high. After *114 hours* the parasites appeared to be much rarer, and the number of dividing forms was considerably less. Many of the parasites, however, still looked healthy. No forms with four chromatin dots were seen at this stage, but on the other hand a considerable number of naked grains like anaplasmas were found in the red cells. From this time onwards the parasites gradually died out in the culture tubes. Flagellated forms were never observed.

In this experiment, although there appeared to be a definite increase in the number of dividing forms in the culture tubes, no stages of development were observed that had not a counterpart in the blood of the ram before incubation. In the tubes the dividing forms at first appeared as compact groups in the red cells (Pl. XXIV, figs. 23 and 26), but later on the chromatin dots became more and more widely separated (Pl. XXIV, figs. 24, 25, 27, 28). Finally very few typical groups persisted, but many of the red cells were found to contain single chromatin dots (Pl. XXIV, fig. 29).

It would appear therefore that, as figured by Nuttall (1913),[1] cross forms, consisting of four chromatin grains, probably represent the stage of maximum division of the parasite.

In addition to the *Theileria* described above, one of the Hausa cattle examined at Calabar was found to be infected with a *Babesia*. The parasites (Pl. XXIV, figs. 30-37) were of a considerable size, and occupied a large part of the cells they infected. The red corpuscles in which they were contained appeared to be somewhat shrunken, and stained more intensely than the uninfected cells. The smallest forms, which were sometimes extracorpuscular, consisted of a large chromatin mass, and a small, almost solid, protoplasmic body. More mature parasites showed a large, and often irregularly shaped body, and a considerable amount of chromatin, sometimes divided into several pieces. What appeared to be segmenting forms, composed as a rule of six or seven chromatin masses, were not uncommon (Pl. XXIV, figs. 36 and 37).

Unfortunately only two imperfect blood films from this animal were available for study, but in these the morphology of this *Babesia* did not seem to correspond with that of any species hitherto described. It is possible, therefore, that it should be regarded as a new species.

One of the dwarf cattle also had a double infection. Besides the minute parasites described above, and provisionally identified as *Theileria parva*, a very few larger forms were found (Pl. XXIV, figs. 38-40). One corpuscle contained two large pear-shaped bodies that occupied the greater part of the cell, so that it is probable that this parasite was *Babesia bigemina;* the disease in this instance occurring in its benign form.

In the greater part of Northern Nigeria horses and cattle are plentiful, as well as the smaller domestic animals. The cattle are of the large humped-back or zebu breed, and are generally referred to as Hausa or Fulani cattle. In Southern Nigeria, on the other hand, horses can only be kept in a few places, and the only cattle to be found are of a dwarf breed. The reason for this difference has been attributed to the denser nature of the vegetation in the south, and the greater prevalence of tsetse flies and the accompanying trypanosomiases. This is no doubt in the main correct, but the observations recorded above suggest that *babesiasis* may be a subsidiary factor.

Southern Nigeria, being without suitable cattle, is largely dependent on Northern Nigeria for its meat supply. In the dry season immense herds of cattle trek south from the provinces of the north. It is well known that but a small proportion of the animals that set out on this journey eventually reach the coast towns. The majority sicken on the road, and have to be slaughtered.

In a former paper (1913)[2] an account was given of the examination of cattle treking through Ilorin on their way to the coast, in which it was shown that very many of them were suffering from trypanosomiasis. The herdsmen are quick to note the first signs of sickness in their stock, and on detecting them at once kill the animals and dispose of the meat at the best price they can obtain. Considerable loss is undoubtedly entailed, as the animals have often to be slaughtered in unfavourable markets.

The blood films obtained from the slaughter-houses at Onitsha and Calabar were from animals that had been bred in Northern Nigeria, and had treked south from that country. Those animals slaughtered at Calabar had, of course, reached the end of their journey, but those killed at Onitsha had not. It is improbable that the latter would have been sacrificed had they not been showing indubitable signs of illness, and the large number of babesias found in their blood suggests that in them these parasites were the cause of a serious disease. Since apparently healthy domestic animals in Southern Nigeria have been found infected with babesias, it is probable that the imported animals from Northern Nigeria became infected in this country, and not possessing the natural immunity of the indigenous stock, were seriously affected.

From time to time it has been suggested to improve the breed of various domestic animals in Nigeria by the introduction of European stock. In any such experiments it will be necessary to guard not only against the tsetse fly that transmits trypanosomiasis, but also against the tick that transmits babesiasis.

BABESIASIS OF GUINEA-PIGS

In this place brief mention should be made of two *babesia*-like parasites occurring in guinea-pigs. Their pathological significance is at present quite obscure, and several animals known to be infected with one or other of them have remained in apparent good health for many months. It should be pointed out, however, that in Nigeria guinea-pigs are liable to die suddenly without apparent cause, and that it is possible that in some of these cases death may be due to the parasites described below. The importance of these bodies is enhanced in view of the extensive use that has recently been made of guinea-pigs in studies relating to yellow fever, as it is necessary to exclude their presence before making use of the animals for experimental purposes.

Paraplasma flavigenum (Pl. XXIV, figs. 41-43).

The first was a parasite, closely resembling some of the forms of *Theileria parva*, which was found in two stock guinea-pigs that had come from Calabar and Aro respectively.

Morphologically it was indistinguishable from *Paraplasma flavigenum* as it appears in the human blood, and in guinea-pigs inoculated from persons suffering from yellow fever. The parasites, as they appeared in the red corpuscles, were composed of a minute chromatin dot, and a rounded or oval protoplasmic body. The protoplasm often enclosed a vacuole, but more minute forms with solid bodies also occurred. In some cases the chromatin was divided into two pieces, but with this exception no other stages of division were seen in the two cases studied. In both the parasites were always rare.

Considering the endemicity of yellow fever in Nigeria, and the probable frequency of the disease in the natives, it is not impossible that these bodies may actually have been due to a natural infection with this disease. The evidence at present available is insufficient to decide this point with certainty; but if these parasites are not in fact *Paraplasma flavigenum*, they must belong to a new species of that genus very closely allied to it.

Paraplasma cobayae, sp. nov. (Pl. XXIV, figs. 44-49).

The second was a parasite morphologically somewhat similar to the first, but differing from it especially in producing stippling of the red corpuscles. It was found frequently in the blood of stock guinea-pigs obtained from Calabar and Aro. Nothing is known as to its pathological significance, but the most heavily infected guinea-pig met with remained healthy during the five months it was under observation, and twice successfully resisted infection after inoculation with human trypanosomes. In this animal the parasites were almost constantly present in the blood, but on some days they were rare, whilst on others they were plentiful.

The youngest form of the parasite that was recognisable consisted of a dot of chromatin, and a minute, solid protoplasmic body. The chromatin was generally greater in amount than occurred in *Paraplasma flavigenum* at a similar stage of development. The largest forms observed were ovoid or ring-shaped, and were definitely vacuolated. All stages between these two types were met with. Occasionally the chromatin was divided; and occasionally it assumed a horseshoe form.

The red corpuscle in which the parasite was enclosed was always more or less stippled. The degree of stippling varied considerably, being very coarse in some cells, and almost imperceptible in others. The difference seemed to depend on the intensity of the staining, and not on any stage of the development of the parasite; the more heavily stained specimens showing the coarser stippling.

This parasite, although closely allied to *Paraplasma flavigenum*, is easily distinguished from that species by the stippling of the corpuscle, and by the somewhat larger and coarser structure of the organism itself As it appears to be a common parasite of the guinea-pig in Nigeria, we propose for it the name *Paraplasma cobayae*.

REFERENCES

1. NUTTALL, G. H. F. (1913). The Herter Lectures. III, Piroplasmosis, Parasitology, Vol. 6, No. 3, p. 302.

2. MACFIE, J. W. SCOTT (1913). Trypanosomiasis of Domestic Animals in Northern Nigeria. Annals of Trop. Med. and Parasit., Vol. VII, No. 1.

A SPIROCHAETE ISOLATED FROM THE BLOOD OF A GUINEA-PIG

This spirochaete was observed in a culture of the heart blood of a guinea-pig at Lagos. Two c.cm. of blood were drawn off with aseptic precautions and added to a sterile tube containing 1/10 c.cm. of a 50% aqueous solution of glucose. The blood was then defibrinated, transferred to a smaller sterile tube, and incubated at 37° C.

On the third day, after forty-six hours' incubation, a few small spirochaetes were found in the culture. They rapidly increased in number, so that by the fifty-third hour there were a considerable number present, and by the seventy-ninth hour they were innumerable. From this time, however, they gradually decreased in numbers, and many of those that were present appeared to be degenerating. The central core broke up at first into short pieces, and then into small dots or granules which were seen to be still enclosed in a faintly-stained sheath. By the eighth day all spirochaetes had vanished. The spirochaetes appeared to be developing in the layer of serum overlying the corpuscles.

At first the spirochaetes were all very minute (Pl. XXV, fig. 22), but later much larger forms appeared. In a film taken after fifty-three hours' incubation fifty individuals, taken as they came, were measured. The middle lines of the spirochaetes were drawn with a camera lucida, and these were measured by the tangent line method. The details of the measurements are shown in Table 2. The parasites ranged in length from 2 μ to 9 μ, and the crest of the curve of measurements occurred at 5 μ. Two individuals, however, measured 14 μ. These were double forms, a type which at this stage was just beginning to appear in the culture tube.

At the same period of incubation, fifty-three hours, the number of undulations in fifty individuals was counted (see Table 3). The smallest number noted was two, and the greatest nine; but the great majority of the spirochaetes had three, four, or five undulations.

Twenty-six hours later, that is after seventy-nine hours' incubation, fifty more spirochaetes were drawn and measured. This was the period at which the parasites were most numerous in the culture. The majority of the spirochaetes were small, measuring

2 μ to 3 μ, or even less; but a few very long forms were also present. The latter appeared to be multiple forms, and were probably the result of incomplete divisions. In a film taken at this stage one spirochaete was found which measured 35 μ.

TABLE 2.—The distribution, according to length, of 100 spirochaetes from a guinea-pig.

Age of the culture	Number measured	Length in microns																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
53 hours	50	...	1	3	11	13	10	4	5	1	...	...	...	...	2	...	...	...	...
79 hours	50	...	14	12	5	5	2	3	1	2	2	...	...	2	...	1	...	1	...
Totals ...	100	...	15	15	16	18	12	7	6	3	2	...	...	2	2	1	...	1	...

TABLE 3.—The distribution, according to the number of undulations, of 50 spirochaetes from a guinea-pig.

Age of the culture	Number measured	Number of undulations									
		1	2	3	4	5	6	7	8	9	10
53 hours ...	50	...	3	13	12	11	6	2	1	2	...

Already after seventy-nine hours' incubation the spirochaetes had begun to undergo marked structural changes. The characteristic undulations were becoming indistinct, and individuals showing knots of chromatic material, beading of the core, and granule formation were common. From this time onwards these changes progressed until the culture died out on the eighth day. Latterly the spirochaetes did not show any clear undulations at all, and often consisted simply of a shadowy sheath enveloping a few chromatic granules (Pl. XXV, fig. 33).

The spirochaetes found after fifty-three hours' incubation probably represented the true form of the parasite, which should therefore be considered to measure 2 μ to 9 μ in length, and to have two to six undulations. The longer forms, and the forms with more undulations, were probably double. The extremities of the spirochaete were usually blunt. When observed with dark ground illumination the spirochaetes were seen to be very active.

Although the spirochaetes rapidly underwent structural changes after the seventy-ninth hour of incubation, they apparently retained their infective power. After 100 hours' incubation a loopful of the serum from the culture was inoculated into the clear fluid that had separated out above the corpuscles in a tube of human blood prepared by Bass' method (i.e., 10 c.cm. of blood to 1/10 c.cm. of a 50% aqueous solution of glucose, defibrinated, and incubated at 37° C.). The human blood in this case was not fresh, but had been prepared and incubated at 37° C. for seven days. The spirochaetes readily multiplied in this medium, and were present in sufficient numbers to be detected after forty-three hours. At this stage they were all very minute, not more than 1 μ to 3 μ in length. After fifty hours these small forms were very numerous, and after sixty-six hours longer forms, and a very few double forms appeared.

The original culture when 150 hours old was used to inoculate a clean guinea-pig, a clean white rat, and three tubes of broth. At this stage the culture contained no normal spirochaetes so far as could be determined, but only forms that had undergone the changes referred to above. All the experiments were negative.

I have to thank Dr. Connal, Director of the Medical Research Institute, Lagos, for very kindly allowing me to make the experiment with the blood of this guinea-pig, which was one of a long series of these animals in which the *Paraplasma flavigenum* of yellow fever had been maintained for several months. The guinea-pig at the time it was killed was in a moribund condition, and was supposed to be dying of yellow fever, and it was in the hope of obtaining a culture of *Paraplasma flavigenum* that the experiment was made. No *Paraplasma* was found in the culture, however, and the appearance of the spirochaetes was quite unanticipated, as the guinea-pig was not known to be infected with these organisms.

Dr. Connal informs me that he had never detected spirochaetes in his routine examinations of the blood of the guinea-pig; and a blood film taken at the time of the experiment showed none of these parasites.

de Gasperi (1912)[3] has also fortuitously isolated a spirochaete, in glucose-lactose gelatine cultures, of the heart blood of a guinea-pig. His spirochaete measured $9{\cdot}6\,\mu$ to $24\,\mu$ in length, and had five, six, or eight undulations. The extremities were pointed. In these respects it differed widely from the spirochaete described above.

As there is no evidence that would suggest an association between this spirochaete and the parasite of yellow fever with which the animal was infected, it must be concluded that it was a specific parasite of the guinea-pig.

REFERENCE

3. DE GASPERI, F. (1912). Présence d'un Spirochète dans le Sang d'un Cobaye. Bull. Soc. Path. Exot., Vol. 5, No. 8, p. 589.

BACILLIFORM BODIES FOUND IN THE RED CORPUSCLES OF A RAT

The curious bodies shown on Pl. XXIV, figs. 50-53, were found in a single brown rat at Ikotobo. The rat when captured was in a moribund condition, and died soon after it was brought to me. A dissection of the body revealed no obvious morbid conditions.

In blood films, stained with Giemsa's solution, a few of the red corpuscles were found with the appearance shown in the figures. They contained a number of rod-shaped bodies, closely resembling bacilli, scattered irregularly in the cells. Some cells showed but a few of these bodies, others were literally packed with them. On minuter examination no special structure could be made out in the bacilliform bodies; they measured roughly $1\,\mu$ by $0{\cdot}25\,\mu$, and stained homogeneously a dark blue or purplish colour. The blood of the rat showed well-marked polychromasia, but it was only in red corpuscles that stained the normal pink colour that these bodies were found. No free forms were seen in the blood, neither did the leucocytes appear to have ingested any of them.

Dr. Connal informs me that Dr. O'Brien found similar bodies in the blood of a giant rat at Accra, on the Gold Coast, and that a note on the subject appeared in the Report of the Accra Laboratory for 1911. I have not had an opportunity of consulting this report, but I understand that Dr. O'Brien observed these bodies in the red cells on several successive occasions, that they gradually increased in number, and that they appeared to be associated with some symptoms of illness.

It is improbable that these bodies could have been due to a remarkable form of basophilia. Their regular shape, and sharply defined outline did not at all suggest such an explanation; and the fact that they only occurred in the normally stained red cells, and not in the polychromatic ones, seemed to be contrary to this view. It is also unlikely that they were bacilli in, or upon, the red cells, as none were found free in the blood, and there was no evidence of their ingestion by leucocytes. The fact that the bodies occurred in considerable numbers in a few corpuscles, and not evenly distributed in most of the red cells, is also against this view. It seems probable, therefore, that they must have been endoglobular parasites, although I am not acquainted with any protist organism at all resembling them.*

A DISEASE OF FOWLS CHARACTERISED BY INCLUSIONS IN THE LEUCOCYTES

The disease described below was observed in five fowls during the months of November and December, 1913. Four of the cases occurred at Eket, and one at Ikotobo. Shortly before my visit to the district, a considerable number of the fowls at Eket had died of this disease, which was well known to the natives, and was considered to be almost invariably fatal.

The symptoms observed were as follows:—The first indication that the fowl was ill was that it showed a tendency to stand stock-still with tail and head drooping. Later it assumed a typical attitude with its shoulders hunched up, its head sunk, its tail depressed, its feathers ruffled, and its eyelids closed. In this attitude the bird would stand indefinitely if undisturbed. As the disease advanced the wings also drooped, and the bird became

---

*The above note was written in Nigeria where I had not the opportunity of consulting recent literature. Since returning to England I have consulted the work of various authors, and especially the recent article by Laveran and Marullaz (Bull. Soc. Path. Exot., Vol. VII, p. 240. 1914), and I believe that without doubt the bodies observed by me were the same as those originally described by Graham-Smith, and subsequently named *Grahamella* by Brumpt. It should be mentioned, however, that forms such as those figured by Graham-Smith in his coloured plate were never seen by me.

unsteady, and unable to stand except by separating its legs to an unusual extent. Finally it lay on the ground, making no attempt to move, until death supervened. Diarrhoea was a marked symptom. Anaemia was not, however, constant, and in two of the cases it was noted that the comb was of a deep red hue, and that the blood was unnaturally dark, and almost chocolate coloured.

The disease was exceedingly acute, and death usually occurred within two days. On examination the body was as a rule neither much emaciated nor conspicuously anaemic. The heart was distended, and in one case the pericardium was full of a greenish fluid; the spleen was somewhat enlarged and soft; and the liver was enormous, pale, and friable. There were no necrotic patches on the liver. The gut was normal.

On microscopical examination the liver was found to have undergone profound fatty degeneration, and the spleen also contained a little fat. No spirochaetes were observed in either sections or smears of the liver and spleen.

The general appearance of the fowls at once suggested that they were suffering from spirochaetosis. The dropping of the head and tail, the somnolence, and the diarrhoea were characteristic of this disease; but the absence of marked anaemia, the fact that paralyses were never observed, and the shorter duration of the disease pointed to some other condition. The most careful examination of blood films taken at every stage of the disease, moreover, failed to reveal the presence of spirochaetes. In every case, however, leucocytes were found enclosing chromatic granules and rings of a type that did not occur in the blood of healthy fowls.

The leucocytes in which the granules were observed were big cells with a large and often kidney-shaped nucleus, and a considerable amount of blue-stained cytoplasm. The granules varied in size from minute points to large spherical masses. They were, however, most frequently large rounded or annular bodies, but not infrequently assumed a club-shaped or signet-ring form. They appeared to consist entirely of chromatin, and in no case was any blue cytoplasm observed attached to them. In blood smears in which the enveloping cell had burst, these granules and rings were seen lying freely on the slides (Pl. XXIV, fig. 65). In smears of the liver and spleen the same bodies were found in great quantities in the cells, but in these organs the granules were often much larger than in the peripheral blood, and so crowded together as to form definite masses (Pl. XXIV, fig. 60).

The red corpuscles also were affected. In the earliest stages of the disease red cells were frequently seen the nuclei of which did not stain the normal deep chromatin tint, but took on a somewhat browner hue with Giemsa's solution. Their cytoplasm was relatively scanty, and tended to assume a brownish colour also. In the latest stages corpuscles of the types illustrated (Pl. XXIV, figs. 55-58), the cytoplasm of which was more or less stippled with brownish or purplish granules, appeared in the blood. It is probable that the dark, almost chocolate colour of the peripheral blood already alluded to was due to the presence of these corpuscles. Similar red cells were seen in smears from the livers and spleens of infected fowls.

In three cases subcutaneous injections with atoxyl were given in the hope that this treatment, which is so successful in the case of spirochaetosis, might prove beneficial. Two of the cases were in the early stage of the disease, and had only just begun to show symptoms. In the blood of the first the chromatic granules described above were present in only a very few leucocytes: this fowl recovered; in the blood of the second the granules were very numerous, and many of the red cells showed the changes already referred to: this fowl died. The third case was moribund at the time of the injection, and died two hours later.

Comparing these results with those obtained with the same drug in spirochaetosis there can be little doubt that its efficacy is much less in this disease. It should be mentioned, however, that a number of fowls living in the same run at Eket as two of the cases were given a prophylactic dose of 3/50 gr. of atoxyl apiece, and that no further cases occurred. It had been anticipated that the disease would spread, as it had done on a former occasion, and kill off the majority of the fowls. It is not certain, nevertheless, that the arrest of the epidemic can properly be attributed to the atoxyl.

No ticks were found on the fowls, but only a few minute red mites on the skin under the wings.

One of the cases was that of a British fowl landed from a steamer at Oron on November 10th, and taken next day to Eket. This bird was first observed to be ill on December 9th, and died the same day. The incubation period in this instance must therefore have been less than a month.

The disease is inoculable. A healthy fowl, in whose blood neither parasites of any sort, nor leucocytes with granules of the type described above could be found, was inoculated subcutaneously with 5 min. of bood from one of the cases. On the fifth day a considerable number of leucocytes containing the characteristic granules were found in its blood. On the sixth day they were less numerous, on the seventh day they were rare, and on subsequent days none were found. No symptoms of illness were observed. This fowl was a native one, and must have been exposed to natural infection all its life, so that it is quite likely that it possessed some degree of immunity to the disease which would account for the transitory nature of the infection. The subsequent history of the fowl would seem to prove that recovery from an attack confers an immunity, for three weeks later it was reinoculated with blood from another case without result.

With the exception of the granules in the leucocytes no other constant feature that suggested itself as of etiological significance was found in this disease which clinically presented a very definite form, and no organisms were detected in the blood and tissues of the fowls to the action of which the condition might be attributed. For this reason it is suggested that these cell inclusions were the specific cause of the disease; an hypothesis which obtains some support from the fact that they were successfully transmitted by inoculation to a healthy fowl.

BLOOD PARASITES OF LIZARDS AND TOADS

During 1913 a number of lizards and toads were examined with a view to determining the blood parasites they harboured. The species of lizard was *Agama colonorum*, and the toad *Bufo regularis*. Altogether thirty-eight lizards and twenty-eight toads were examined; and of these fifteen and twelve respectively were found to have parasites in their blood. Multiple infections were very common. In the blood of one lizard, for example, two species of micro-filaria, a trypanosome, a haemogregarine, and a haemocystidium were found.

With the exception of one lizard which came from Ilorin, and eight examined at Ikotobo, all the animals were caught in the grounds of the Medical Research Institute, Lagos.

TABLE 4.—Parasites found in the blood of toads and lizards in Nigeria

	Toad (*Bufo regularis*)	Lizard (*Agama colonorum*)
Number examined......	28	38
Trypanosomes	4	1
Microfilariae	3	13
Haemogregarines	8	2
Haemocystidia	...	3

LIZARDS (*Agama colonorum*).

Of the thirty-eight lizards examined, fifteen were found to have parasites in their blood. Nine had a single infection, and six had a double or multiple infection. Trypanosomes were found in one individual, micro-filariae in thirteen, haemogregarines in two, and haemocystidia in three. The largest number of parasites was always found in the oldest lizards, the most heavily infected being males whose stunted tails and deformed claws seemed to indicate that they were of a considerable age. So far as could be determined, the lizards did not appear to suffer from the parasitic infections. A number of heavily infected individuals were kept in captivity for several months, and appeared to enjoy good health as long as they were under observation.

Trypanosomes (Pl. XXV, fig. 12).

In a single male obtained from Ilorin, Northern Nigeria, broad stumpy trypanosomes were found in the blood. The parasites were extremely scanty, only one being seen in a fresh preparation, and one in a stained blood film.

In the fresh preparation the trypanosome exhibited rotatory movements, but did not swim actively about the field.

The single individual found in the stained film measured 40 μ in length, and about 11 μ in breadth at its widest part. The morphology of the parasite can best be made out from the accompanying sketch (Pl. XXV, fig. 12). The nucleus, which was indistinct, was situated about the middle of the body; and towards the blunter end of the trypanosome there appeared to be a small blepharoplast. The flagellum was attached to the body by a relatively scanty membrane, and there did not appear to be any free portion, or only a very short one, projecting beyond the body.

No figure of a trypanosome exactly similar to this one has come under our notice, but Todd and Wolbach (1912)[4] have described a form in a lizard of this species from the Gambia which was possibly the same.

Filarial embryos

Filarial embryos were present in the blood of thirteen of the lizards examined. They were of two distinct types, which for convenience we may term the slender, and the stumpy forms. In six lizards both forms were present, and in all these enormous numbers of the embryos were found, so that on observing the fresh blood with the microscope, it seemed to consist of little besides a writhing mass of parasites.

The identity of these filariae has not yet been determined, and it would therefore be superfluous to give a detailed description of the embryos found in the blood. Brief mention should, however, be made of them since they form such a striking feature of the blood picture of the lizards in Nigeria.

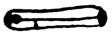
Slender type (Pl. XXV, fig. 1).

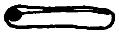
Long, slender filarial embryos were found in twelve lizards. The general form of these parasites is shown in the figure (Pl. XXV, fig. 1). They were enclosed in a sheath which was exceedingly difficult to stain, but within which the embryos could be seen actively moving in fresh preparations of the blood.

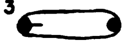
The average length of twenty individuals, taken as they came, and roughly drawn with the aid of a camera lucida, and measured by the tangent line method, was 129 μ. The longest was 154 μ, and the shortest 106 μ. Embryos measuring as much as 168 μ at any rate were occasionally seen. The breadth was about 4 μ at the widest part.

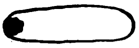
The tail was sharp, but the cephalic end was rounded. There was always a clear area of about 4 μ at the cephalic end. The most constant gaps and breaks in the body occurred, in an embryo of the average length of 129 μ, at 37 μ, 54 μ, 83 μ, 113 μ, and 124 μ from the cephalic end; or in approximate percentages of the total length of the parasite, at 29, 42, 64, 88, and 96 % respectively. As the lengths of the breaks and gaps varied considerably the above figures are calculated to the middle points of the clear areas in each case.

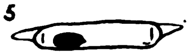
Associated with these micro-filariae, small dumb-bell shaped bodies, about 12 μ long, were invariably found in the blood (Pl. XXV, fig. 4). In intensely stained films these bodies were seen to be surrounded by an irregularly-shaped envelope composed of a material that appeared to be similar to that of the sheaths of the filarial embryos. The association of these bodies with the micro-filariae was not at first suspected. It was noticed, however, that lizards in whose blood at first these bodies alone were seen, later on

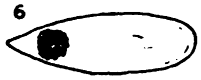
1


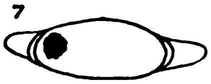
2


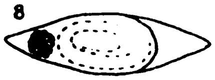
3


4


5


6


7


8


were found to be infected with the slender type of filarial embryos, and finally in the uteri of adult filariae from the mesentery both dumb-bell shaped bodies and immature filarial embryos of the slender type were found together. All stages between a simple rod-shaped body with a darker dot at each end and a thin connecting line, and filarial embryos such as appeared in the blood were found. The earlier stages of this development are illustrated by the figures in the text (figs. 1-8), which are sketches drawn with the aid of a camera lucida from a preparation of the uterine contents of one of the filariae, and arranged in what may be supposed to be their proper sequence.

The adults of these filarial embryos were slender, whitish worms found lying between the folds of the mesentery.

Stumpy type (Pl. XXV, fig. 3).

The stumpy type of embryo was found in seven lizards. It was enclosed within an ample sheath which stained much more easily than that of the slender type. The embryos were relatively short and stout; the tail was pointed, but the cephalic end was somewhat blunt. In fresh films their movements were sluggish. The general form of the parasite is shown in the figure (Pl. XXV, fig. 3). In blood that had been shed for some little time many of the embryos of this type were found to have cast off their sheaths, and to be lying free in the plasma.

The average length of twenty individuals, taken as they came and measured by the tangent line method, was 65 μ. The longest embryo measured 78 μ, and the shortest 56 μ. The average breadth, at the widest part was 5 μ-6 μ. At the cephalic end there was a clear area extending for 3 μ-4 μ. The chief breaks in the body occurred at 49 μ and 56 μ from the cephalic end respectively in an embryo of the average length of 65 μ. The position of the breaks was calculated from the middle of the clear area in each case. In some embryos the breaks were represented by two smaller gaps which together stood in place of the larger single gap of the more typical individuals.

The adults were of a bright yellow colour. They were found in the subcutaneous connective tissues all over the body, and were often arranged in close coils or knots, consisting as a rule of a male and a female, in situations where the skin was loose, or were found running down between the muscles of the back on either side of the vertebral column. In the uteri of the females, embryos were seen similar to those found in the peripheral blood of the lizards.

Haemogregarines (Pl. XXV, figs. 16-21).

In the blood of two lizards, the one from Ilorin and the other from Ikotobo, haemogregarines were found.

Both endoglobular and free forms were present. The latter were rare; but in one of the lizards the former were plentiful. The free forms (Pl. XXV, fig. 20) were rather slender bodies with one end slightly blunter than the other. At this end, lying close to the nucleus, an oval area that stained in a distinctive manner was usually seen to be differentiated in the cytoplasm. The endoglobular forms were enclosed by a definite capsule. They varied in shape from almost spherical bodies to elongated and hoop-shaped forms occupying the whole length, or the whole of one end, of the corpuscles. The corpuscles themselves were not greatly distorted by the parasites, and reacted to staining reagents in a normal manner. The parasites lay within the capsules in a more or less bent position. In the spherical forms they were bent double; but in the more commonly observed elongated forms they lay with their extremities turned only slightly inwards towards the nuclei of the corpuscles.

A considerable number of chromatin granules were always scattered about the endoglobular parasites. They were sometimes fine, and generally distributed, but more often they were coarse and tended to be aggregated in masses at the extremities of the capsules or along their borders. No pigment was present.

An interesting point was the fact that a number of the leucocytes were found to contain haemogregarines (Pl. XXV, fig. 21). The parasites appeared to be enclosed in capsules, and stained similarly to those seen in the red cells. Their appearance did not suggest that they had been ingested and were undergoing a process of digestion.

Parasites of this type, occurring in white cells, have been found in the blood of small mammals and vultures, and have been named *Leucocytogregarina*. The organism described above appears to be peculiar in that it is found both lying freely in the blood, and invading the red and white corpuscles, and would appear to be similar to the drepanidium found by Dutton, Todd, and Tobey in frogs and toads.[5]

Haemocystidia (Pl. XXV, fig. 8).

In three lizards pigmented parasites were found in the red blood corpuscles which should, probably, be assigned to the genus *Haemocystidium* (Pl. XXV, fig. 8). In three other lizards pigmented leucocytes were detected, so that it is possible that had more material been available pigmented parasites might also have been found in their blood.

Todd and Wolbach, in the paper referred to above, recorded the occurrence of 'A pigmented haemocytozoon in a lizard (*Agama colonorum*)' which may have been the same parasite; and Wenyon (1908)[6] has described under the name *Haemoproteus agamae* a pigmented parasite in the same species of lizard from Wau, in the Bahr-el-Ghazal. 'The affinities of this parasite,' he says, 'are evidently with those of halteridium, from which it differs, however, in the shape of the gametocytes and, perhaps, in the fineness of the pigment grains.' It is probable that the parasites from Nigeria should be referred to this species.

TOADS (*Bufo regularis*)

Nine of the twenty-eight toads examined were infected with a single species of parasite, and three with two or more. Trypanosomes were found in four, filarial embryos in three, and haemogregarines in eight. A considerable number of the toads examined were quite young animals; but the general impression formed was that the majority of adults is infected with haemogregarines. Trypanosomes and micro-filariae were relatively much rarer, and were always rather uncommon in the blood.

Trypanosomes (Pl. XXV, figs. 10, 11, 13-15).

Three species of trypanosomes were met with in toads, namely, *T. mega*, *T. rotatorium*, and another form which is possibly a new species.

In one toad a trypanosome was found that was apparently identical with *T. mega* (Pl. XXV, fig. 13). The parasite was always scanty in the blood, and in spite of its large size was exceedingly difficult to find. During the first twenty days the animal was under observation, however, it was constantly present in the peripheral blood, and no other forms were ever met with in this animal. The occurrence of this trypanosome in the toad has previously been recorded by several authors.

An interesting observation was made on this parasite. On April 23rd, 1913, the toad was placed in an incubator at a temperature of 38° C. It was thought possible that the unnatural conditions thus produced might enable the trypanosomes to gain an advantage over the host, and that they might become more abundant in the blood. Forty-two hours later the toad was removed to the ordinary temperature of the laboratory. It appeared to have been adversely affected by the temperature of the incubator, as two blisters had formed, one on either side of its back. On the same day one degenerated trypanosome was found in the blood, but with this exception no trypanosomes were detected during the subsequent 120 days the animal continued to be under observation.

That the trypanosomes did not multiply although the host was obviously affected may have been due either to the effect of the unusually high temperature on the trypanosomes themselves, or to the fact, which has been suggested, that the parasites found in the blood are in a latent stage awaiting an opportunity for further development in another host, but not for the time being undergoing multiplication. In this connexion it is interesting to contrast the experiments of Wendelstadt and Fellmer (1909)[7] on the vitality of mammalian trypanosomes in cold-blooded vertebrates. In recording the results of inoculations with *T. lewisi* they stated that temperature had an influence on the infected cold-blooded animals. Adders and lizards stood *T. lewisi* infection well at room temperature, less well at 37° C. If, as may be supposed, this lowered resistance was due to some diminution in the vitality of the cold-blooded host, the experiment described above with *T. mega* and *Bufo regularis* would seem to indicate that the change of temperature had an even greater prejudicial effect on the vitality of the trypanosome.

Three toads were found to be infected with *T. rotatorium* (Pl. XXV, figs. 10 and 11). The parasites were morphologically identical with those figured in the Fourth Report of the Wellcome Tropical Research Laboratories, and identified there as *T. rotatorium;* and with the figure of *T. sanguinis* from frogs given by Dutton and Todd in their First Report of the Trypanosomiasis Expedition to Senegambia (1902). In only one of the three toads were the parasites at all numerous.

In fresh preparations the ample membrane of the trypanosome was seen to be in constant, and very active motion. The average length including the free portion of the flagellum was 35 μ.

In a single toad, which also harboured *T. rotatorium*, another form of trypanosome was found (Pl. XXV, figs. 14 and 15). This parasite measured from 63 μ to 74 μ in length, and had a long free flagellum. The nucleus was indistinct, the blepharoplast was, on the other hand, large and prominent. The cytoplasm stained almost uniformly blue, but merged insensibly with the membrane, which was ample and deeply folded. The posterior end of the trypanosome was prolonged for a considerable distance beyond the blepharoplast, and gradually tapered to a point. It was not uncommon to find these parasites, in stained preparations, coiled into a ring or spiral.[*]

The general appearance of the parasite is illustrated in the accompanying plate. We are not aware that any similar trypanosome has previously been recorded from a toad (*Bufo regularis*).

A few attempts were made to transmit *T. mega* and *T. rotatorium* by inoculations. Two lizards were injected, intraperitoneally, with blood containing *T. mega* from the toad. Neither developed trypanosomiasis. Two lizards and four toads were inoculated similarly with toad's blood containing *T. rotatorium*. None of these animals became infected. The lizards remained under observation for twenty-two days, and fifty-four days; and the toads for fifty days, twenty-nine days, and fifty days, respectively.

No dividing forms of any of the above trypanosomes were seen in the peripheral blood.

Filarial embryos

Filarial embryos (Pl. XXV, fig. 2) were observed in three of the twenty-eight toads examined. They were in each case very rare, and it was not possible to find a sufficient number on which to base a study of their morphology. The general form of the parasites is, however, shown in the figure. The length was about 110 μ, and the breadth 4 μ-5 μ. There was no sheath.

The behaviour of these parasites in the circulating blood was watched in the vessels of the mesentery, but beyond the fact that they passed along in very actively wriggling coils, and were never seen fully extended, no particular observations were made. Anastomoses between the smaller blood vessels of the mesentery are numerous, and it was not therefore necessary for the microfilariae to enter the minute capillaries. Indeed they were observed on several occasions to be arrested at the mouth of a capillary arising from a larger vessel until a current of blood of sufficient force swept by to carry them along the broader channel.

---

[*] Compare Dutton, J. E., Todd, J. L., and Tobey, E. N. 'Concerning certain Parasitic Protozoa observed in Africa,' Annals of Trop. Med. and Parasit., Vol. V, p. 327, and Pl. XXVIII, figs. 43 and 44, showing the coiled forms of a 'trypanosome resembling *T. karyozeukton*.'

The adult worms occurred in the mesentery attached to the lower third of the gut.

Haemogregarines (Pl. XXV, figs. 5 and 9, 6 and 7).

Haemogregarines are exceedingly common in the blood of adult toads (*Bufo regularis*) in Nigeria, but they are apparently less frequently present in young animals. The accompanying figures (Pl. XXV, figs. 5, 6, 7, and 9) illustrate the appearance of these parasites. The parasites are large, and occupy a considerable part of the red cells, so that the nucleus is much displaced. Large recurved vermicules are commonly seen, and straight or slightly bent forms of various sizes. In some cases merozoite-like bodies were found free in the plasma. The haemogregarines were enclosed in capsules, at the ends of which small collections of chromatin-staining material were sometimes visible.

The parasite is probably of the same species as that found in *Bufo regularis* in Khartoum, and identified by Stevenson (1911)[8] as *H. tunisiensis*.

REFERENCES

4. Todd, J. L., and Wolbach, S. B. (1912). Parasitic Protozoa from the Gambia. Journ. of Med. Res., Vol. 26, No. 2. Quoted in the Tropical Diseases Bulletin.
5. Dutton, J. E., Todd, J. L., and Tobey, E. N. Annals Trop. Med. & Parasitol. Vol. I, p. 331.
6. Wenyon, C. M. (1908). Third Report of the Wellcome Research Laboratories, p. 148.
7. Wendelstadt, H., and Fellmer, T. (1909). Zeits. f. Immunit, pp. 422-432.
8. Stevenson, A. C. (1911). Fourth Report of the Wellcome Tropical Research Laboratories, p. 360.

THE OCCURRENCE OF A SPIROCHAETE IN THE GUT OF *GLOSSINA TACHINOIDES*

In the course of some experiments with trypanosomes a number of tsetse flies were dissected at Ikotobo in December, 1913, and examined for flagellates. In the lower half of the gut of one *G tachinoides*, a female, innumerable spirochaetes were found. The fly had been fed for some days on a clean guinea-pig, but although the blood of this animal was repeatedly examined, no spirochaetes were ever found in it.

In the fresh preparation the gut was seen to be packed with a writhing mass of spirochaetes. The organisms appeared to move with great activity. When stained, the spirochaetes were mostly found to be aggregated into clumps and tangles, in which it was impossible to follow the individual parasites, but numerous isolated specimens were also present, and a number of these was drawn and measured. Their lengths varied greatly, both long and short forms being found; the former measuring 15 μ to 35 μ, and the latter 7 μ to 10 μ. The spirals were ill-defined and variable, but the longer individuals generally showed five or six distinct undulations, and the shorter ones two or three. The extremities of the spirochaetes were blunt, and the parasites were extremely slender.

Novy and Knapp (1906)[9] have described a spirochaete occurring in the stomach of *G. palpalis*, and have named it *S. glossinae*. They describe it as occurring as single short forms measuring 8 μ in length, and double forms about 15 μ long. The ends of the spirochaete were indistinct and tapering. The short forms had four turns, but the authors noted that in some instances the spirals were drawn out into rather straight forms. The parasite found in *G. tachinoides* at Ikotobo would appear therefore to be distinct from this species.

Nothing is known as to the further history of this parasite. The guinea-pig on which the fly had fed repeatedly did not develop spirochaetosis, and subsequent experiments, in which wild *G. tachinoides* were fed on fowls, were negative. The tsetse appeared to be unaffected by the infection, and had been kept in captivity for twenty-two days before being killed for examination.

REFERENCE

9. NOVY, F. G., and KNAPP, R. E. (1906). Journ. of Infect. Diseas., Vol. 3, p. 385.

EXPLANATION OF PLATES

PLATE XXIV

All the figures were drawn from preparations stained with Giemsa's solution. The blue coloration of the corpuscles in figs. 1 and 2 was due to restaining after the slides had been exposed to the vapour of creosote.

Figs. 1-29. Babesia of sheep and cattle, probably *Theileria parva.* × 2,000. Figs. 1 and 2 from a sheep at Lagos; figs. 3-6 from a sheep at Onitsha; fig. 7 from a dwarf cow at Ikotobo; figs. 8 and 9 from a Hausa cow at Calabar; and figs. 10-19 from a ram at Lagos. Figs. 20-29 from cultures of the blood of the ram at Lagos.

Figs. 30-37. *Babesia sp.* from a Hausa cow at Calabar. × 2,000.

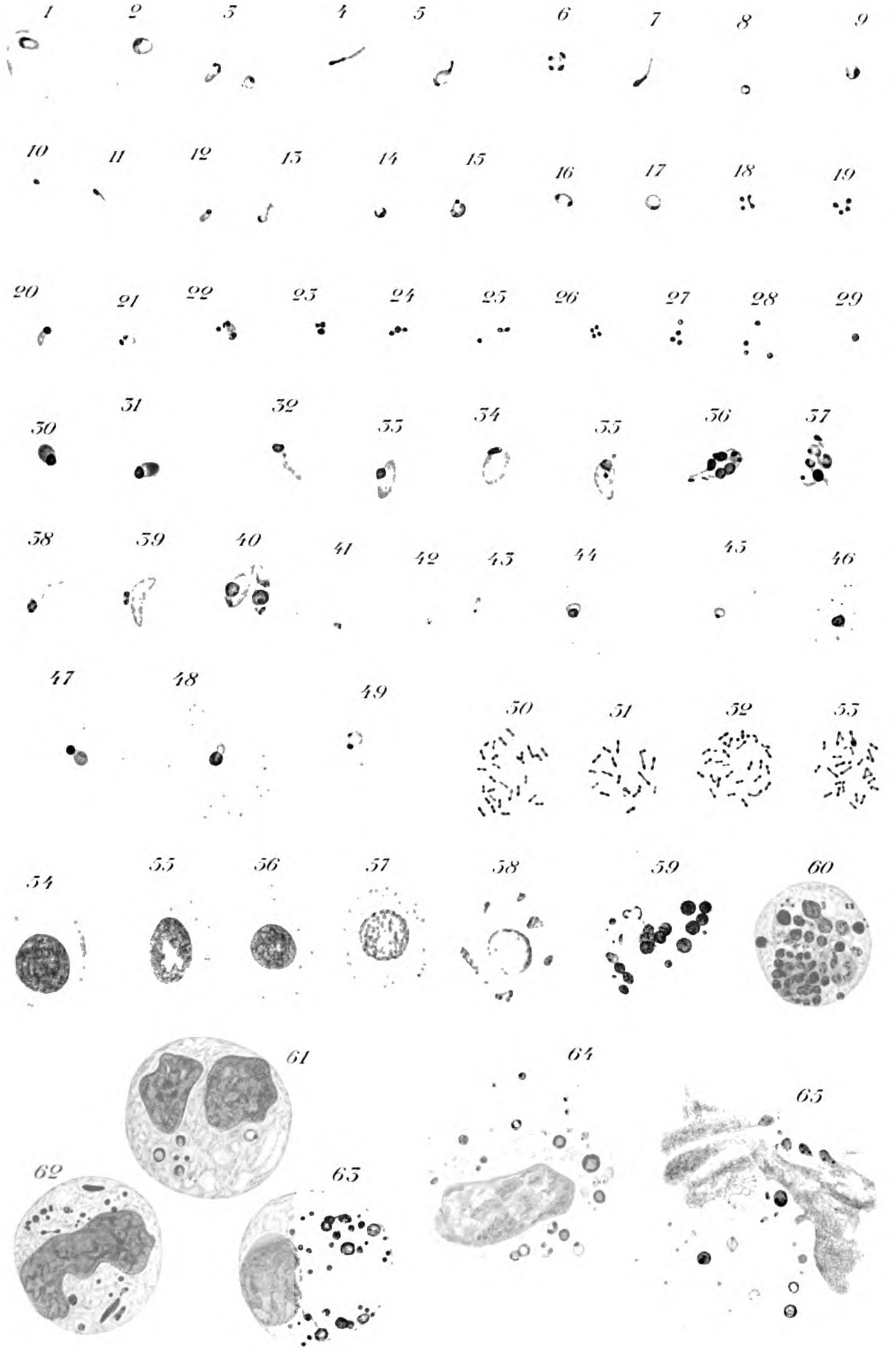
Figs. 38-40. Babesia from a dwarf cow at Ikotobo, probably *B. bigemina.* × 2,000.

Figs. 41-43. Babesia of guinea-pigs closely resembling *Paraplasma flavigenum.* × 1,000.

Figs. 44-49. Babesia-like parasite of guinea-pigs—*Paraplasma cobayae sp. nov.* × 2,000. Fig. 46 depicts one of the naked chromatin masses not infrequently seen which may, or may not be a stage in the development of the parasite.

Figs. 50-53. *Grahamella* from a brown rat at Ikotobo. × 2,000.

Figs. 54-65. Blood corpuscles from sick fowls at Eket and Ikotobo, × 2,000. Figs. 54-58, red blood corpuscles showing the stippling, and the atypical staining of the nuclei. Figs 59-64 leucocytes, fig. 59 from the spleen, fig. 60 from the liver, and figs. 61-64 from the blood, showing the characteristic granules and rings. Fig. 65 a leucocyte which has burst and liberated the granules.



M. Rhodes pinx.

[illegible]

BLOOD PARASITES COLLECTED IN
NIGERIA

PLATE XXV

All the figures were drawn from preparations stained with Giemsa's solution.

Fig. 1. Slender type of filarial embryo found in lizards (*Agama colonorum*). × 1,000.

Fig. 2. Filarial embryo found in toads (*Bufo regularis*). × 1,000.

Fig. 3. Stumpy type of filarial embryo found in lizards (*Agama colonorum*). × 1,000.

Fig. 4. Dumb-bell shaped body found associated with the slender type of filarial embryo (fig. 1) in lizards (*Agama colonorum*). × 1,000.

Figs. 5, 6, 7, and 9. Haemogregarine of toads (*Bufo regularis*). Figs. 5 and 9 large endoglobular forms. × 2,500. Figs. 6 and 7 merozoite-like and free forms. × 2,000.

Fig. 8. Haemocystidium, probably *Haemoproteus agamae*, of lizards (*Agama colonorum*). × 2,000.

Figs. 10 and 11. *Trypanosoma rotatorium* found in toads (*Bufo regularis*). × 1,500.

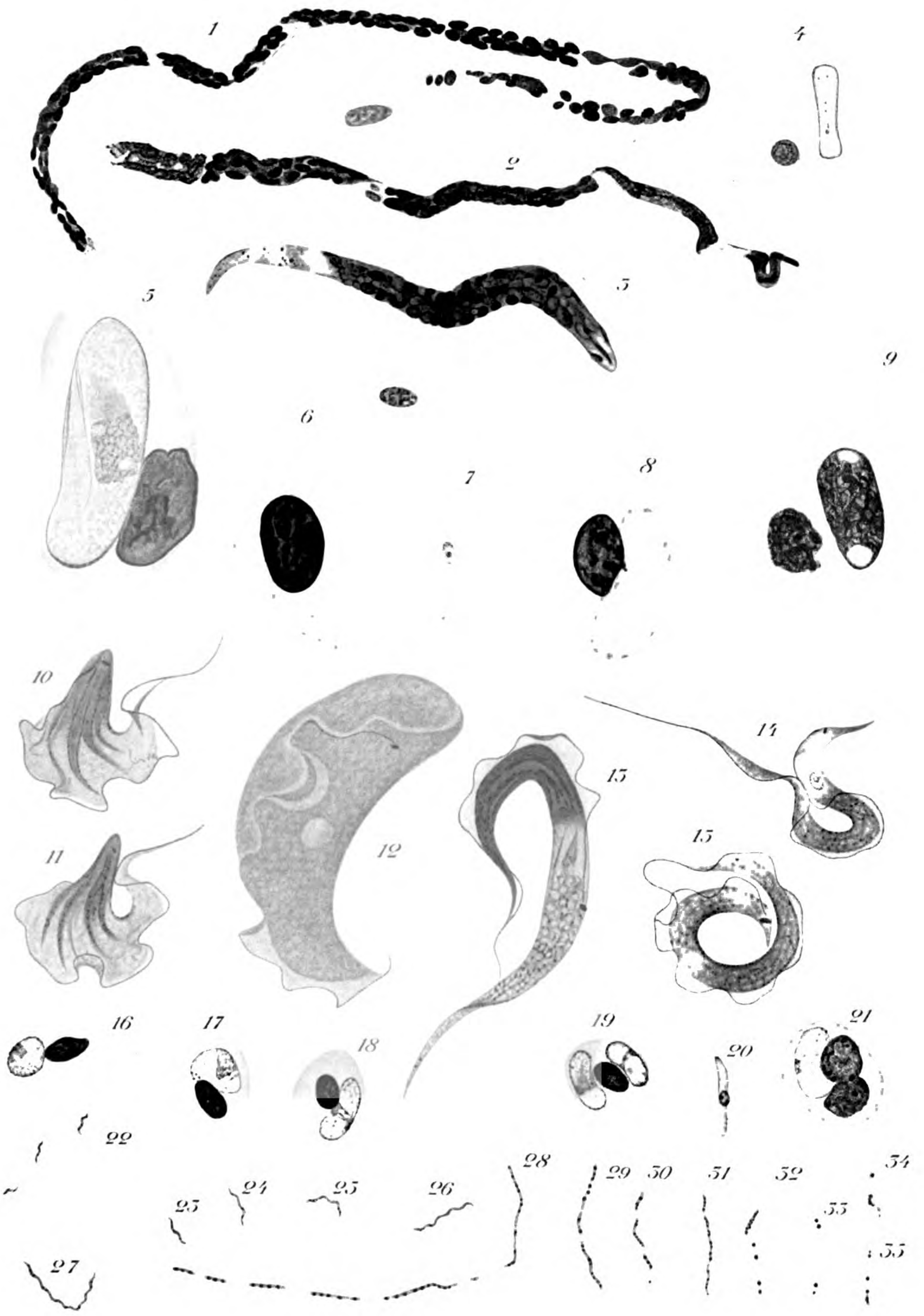
Fig. 12. *Trypanosoma sp.* found in a lizard (*Agama colonorum*). × 1,500.

Fig. 13. *Trypanosoma mega* found in toad (*Bufo regularis*). × 1,500.

Figs. 14 and 15. *Trypanosoma sp.* found in a toad (*Bufo regularis*). × 1,500.

Figs. 16-21. Haemogregarine found in lizards (*Agama colonorum*). × 1,000. Figs. 16-19 in red corpuscles, fig. 20 free, and fig. 21 in a leucocyte.

Figs. 22-35. Spirochaetes isolated in cultures of the blood of a guinea-pig. × 2,000. Fig. 22 small forms. Fig. 28 one of the very long forms. Figs. 29-35 forms showing the breaking up of the core.



M. Rhodes pinx. [illegible]

BLOOD PARASITES COLLECTED IN
NIGERIA

THE TRYPANOSOME OF GAMBIAN MICE

BY

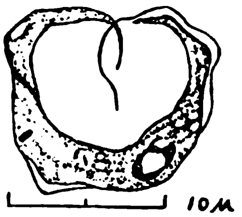
JOHN L. TODD, M.D.

(*Received for publication* 14 *April*, 1914)

In 1902 flagellates were seen in the blood of Gambian house mice (sp. ?).[1] In 1905 *Trypanosoma duttoni* was described in the blood of mice (*Mus morio*),[8] caught in Senegal.[2,3] Since then trypanosomes have been found in mice in several parts of the world.[4,5,6,7]

The flagellate originally recorded from the Gambia was only seen in fresh preparations and it was said to lack an undulating membrane. Recently, dried smears of the blood, in which these flagellates were seen, have been carefully re-examined, and in one preparation two trypanosomes have been found; their size approximates to the dimensions given for the organisms seen in fresh preparations in 1902. Although only two parasites have been seen, they are so nearly identical in size and morphology that it is justifiable to describe them as members of a single species.

Its size and the very long distance between its blepharoplast and posterior extremity are the most striking characteristics of this trypanosome (Text-fig.). The blepharoplast is rod-shaped; the nucleus stains lightly and contains a small amount of loose-textured granular chromatin. At the blepharoplast arises the flagellum which borders a rather scanty undulating membrane; it is continued at the anterior extremity of the parasite as a free



× 2000. Drawn with Zeiss micrometer, Ocular 3, objective 2 mm.

flagellum. The cytoplasm of the trypanosome is finely alveolar; immediately posterior to the nucleus the alveoli seem to be somewhat coarser than elsewhere. By very careful focussing, it can be distinctly seen that the trypanosome is striated lengthwise, and that the striations run diagonally where the parasite is twisted on itself. Its dimensions are, about: (i) posterior extremity to centre of blepharoplast, 14·9 μ; (ii) blepharoplast to posterior border of nucleus, 7·5 μ; (iii) posterior to anterior border of nucleus, 3 μ; (iv) anterior border of nucleus to anterior extremity of cytoplasm, 11 μ; (v) anterior extremity of cytoplasm to free end of flagellum, 6·5 μ; (vi) greatest breadth, without undulating membrane, 3·5 μ; (vii) total length, 43 μ.

In general appearance and in its dimensions this trypanosome much more nearly resembles *Trypanosoma acomys* (Wenyon)[8] than any of the trypanosomes which have been found in the blood of mice.

It is concluded that the haemoflagellate seen in Gambian mice in 1902 is not a herpetomonad parasite, but a trypanosome, and that this trypanosome is possibly *T. acomys*.

REFERENCES

1. Dutton, J. E., and Todd, J. L. First Report of the Trypanosomiasis Expedition to Senegambia. Liverpool School Trop. Med., Memoir XI, 1902, p. 57.
2. Thiroux, M. Ann. de l'Inst. Pasteur, T. XIX, Sept. 25, 1905, pp. 564-572.
3. Thiroux, M. Compt. rend. Soc. de biol., Series 57, Tome I, 1905, p. 885.
4. Kendall, Arthur I. Jour. Infect. Dis., Vol. 3, No. 2, Apr. 6, 1906, pp. 228-231.
5. Pricolo, A. Centralb. f. Bakteriol., 1 Abt. Originale, Bd. XLII, Heft 3, p. 231.
6. Wenyon, C. M. Third Report Wellcome Research Laboratories, 1908, p. 143.
7. Finkelstein, N. I. Archives Sc. biol., T. XIII, fasc. 2, edit. fr.
8. Laveran, A., and Mesnil, F. Trypanosomes et Trypanosomiases, 1912.

THE GRANULE PHASE OF SPIROCHAETES

BY

H. B. FANTHAM, D.Sc., B.A.

(*Received for publication* 29 *July*, 1914)

CONTENTS

I. Introduction 471
II. Spirochaetal Granules—what they are and how they are formed 471
III. Generalisations made from the Granule Phase of Spirochaetes 472
IV. The Spirochaetal Infective Granule and Analyses of the Criticisms thereon 474
V. Further Recent Researches showing the Relation of Granules in the Life-Cycle of Spirochaetes 479
VI. Conclusions 481
References 483

I. INTRODUCTION

Since the classical researches of Leishman in 1909-10 on the life-cycle of *Spirochaeta duttoni* in *Ornithodorus moubata*, first outlined by Dutton and Todd in 1907, much work has been done on the life-history of spirochaetes, those difficult organisms around which controversy has raged for the last ten years. An important phase was the discovery of the granule stage in spirochaetes, around which recently much of the discussion has centred. It is intended in the present paper to endeavour to throw light on the discussion and to record some recent personal results obtained, more especially, while working in Khartoum last year.

II. SPIROCHAETAL GRANULES—WHAT THEY ARE AND HOW THEY ARE FORMED

Leishman's results essentially were that spirochaetes gave rise by multiple fission to granules or coccoid bodies inside the invertebrate host, and that these coccoid bodies or granules found their way more especially to the Malpighian tubules, gonads, and other organs of the tick. The granules themselves multiplied. The eggs of the female tick became infected with granules, and the progeny of infected females might be born infected.

The observations of Leishman have been confirmed and extended by Balfour (1911), Fantham (1911), and Hindle (1911). The spirochaetes investigated more particularly were *S. duttoni* and *S. gallinarum* (or varieties of the latter). These results have been controverted by Marchoux and Couvy (1912-13), by Blanc (1911), and by Wolbach (1914).

That spirochaetes divide by multiple transverse fission into small portions—the granules, coccoid bodies or spores of various authors—really is not open to controversy. Such formations have also been seen in the larger spirochaetes (e.g., *S. balbianii* and *S. anodontae*) found in the digestive tract and crystalline style of Lamellibranch or bivalve molluscs, such as the oyster, cockle, scallop, fresh-water mussel, and allied forms. These molluscan spirochaetes have been observed to divide by multiple fission into spores, comparable with the granules of the blood forms, by Bosanquet (1911), Fantham (1911), and Gross* (1912). The molluscan spirochaetes do not appear necessarily to need an alternate host for their cross-infection.

It may be mentioned that, even as early as 1882, Zopf recorded and figured the formation of micrococcoid and bacillary forms from spirochaetes of stagnant water and of the teeth, and stated that these forms stand in genetic relationship to one another.

The formation of granules or spores in *Treponema pallidum* has been recorded by Balfour (1911) and by Hoffman (1912). These granules have been found, then, in representatives of all the chief members of the spirochaete group, illustrating the importance of considering this difficult subject from the broad standpoint of comparative morphology.

III. GENERALISATIONS MADE FROM THE GRANULE PHASE OF SPIROCHAETES

Unfortunately, for the last two or three years much has been heard of the 'infective granule' in Protozoa. This phrase apparently is due to Balfour. The excellence of the work of that investigator on Sudan avian spirochaetosis and on granule shedding in *Treponema pallidum* cannot be gainsaid, but unfortunately the idea of the 'infective granule' in the Protozoa vera is less happy and has been extended in a manner that is almost harmful. We have had to submit to stories of the infective granule in trypanosomes, in Leishmania, and in haemogregarines, to mention only a few, and we have been treated to speculative inferences about the phylogenetic significance of such a granule, and that in it may be seen the 'primordial granule,' and even the origin of life. However, behind the term 'infective granule,' used thus, lies an assumption and a fallacy, namely, an assumption that the said granule is infective, and a lack of proof that actually it is so. On the other hand, it is well known that trypanosomes and similar organisms give off granules, excretory in nature, during the course of their life, especially while being observed under dark-ground illumination, a paraboloid condenser being used. The stages between a so-called infective granule and the Leishman-Donovon body, or the rounded (latent) stage of a trypanosome, or a vermiform haemogregarine, have not been satisfactorily traced. Until further evidence is adduced, it would be better to call such a granule, if it is to receive a designation, an excretory granule.

---

* In this paper the author revives an old idea that the Spirochaetes are multicellular. He finds that they separate into spores. As the rest of the paper consists of misrepresentations and abuse of the work of other investigators, particularly British, who do not agree with him, the contents can be ignored.

From these remarks concerning the so-called infective granule in trypanosomes, it will be gathered that such granules can occur, according to the upholders of the hypothesis, in the cycle in the vertebrate host. Granules are also apparently considered to be an integral phase in the life-cycle of spirochaetes occurring in vertebrate blood. That granules can occur *inside* spirochaetes while parasitic in various fluids in the vertebrate host, there is no doubt. Such granules can be seen in *Spirochaeta** *bronchialis* while swimming in tracheal expectoration when examined by the aid of the paraboloid condenser, and I often noticed them while working on the subject in Khartoum in 1913. They can be seen also in fixed and stained preparations, treated according to the most approved cytological methods, such as fixation in Bouin's fluid and staining with haematoxylin. In blood spirochaetes the granules are seen with greater difficulty, though I have observed them occasionally in

---

* The generic name *Spirochaeta* is used without prejudice. Until Zuelzer's (1911) researches on *S. plicatilis* are confirmed, and there is more agreement about the life-cycles of these organisms, it is useless to spend much time on provisional classifications of the same.

S. recurrentis (var. *berbera*) in Khartoum. Balfour has described an intracorpuscular phase of *S. granulosa* in bird's blood in the Sudan. When commenting on this phase in 1911, I wrote (p. 484) that 'multiple transverse fission is scarcely an essential phase of the spirochaete in the vertebrate host, but may occur at the crisis, and may explain the "after phase." I regard the phenomenon largely as an anticipation of what occurs in the invertebrate host, which is frequently a tick.' Balfour (1912), in noting this remark, disagreed and indulged in an unfortunate generalisation that 'Nature is not wasteful.' I think that several examples could easily be given of the fallacy of this poetic generalisation—it will suffice to mention the wastefulness of Nature in the phenomena of spermatogenesis and in the production of microgametes among the Coccidia. Further, malarial gametocytes may be produced in a patient's blood, but for their main, natural, progressive development they must find their way into a mosquito. I do not wish to question the importance of Balfour's interesting work in seeking to elucidate the intracorpuscular bodies seen by him in the blood of birds infected with Sudanese spirochaetosis. I cannot, from personal observations, go so far as he does in interpreting the intracorpuscular bodies in question. I believe, however, that it is highly probable that spirochaetal granules are connected with relapses when such occur in spirochaetosis in the vertebrate host. In connection with Balfour's work (1911) on granule shedding in *Treponema pallidum* I wrote (p. 493) that 'the granules of *T. pallidum* may explain the *Cytoryctes luis* of Siegel.' Also I think that the granules are more resistant to drugs than the spirochaete forms, and in this way are responsible for relapses, sometimes long deferred.

The real granule phase of spirochaetes, which occurs in the invertebrate host of those possessing a natural alternation of hosts, may now be considered.

IV. THE SPIROCHAETAL INFECTIVE GRANULE AND ANALYSES OF THE CRITICISMS THEREON

According to the work of Leishman, Balfour, Fantham and Hindle on *S. duttoni* and *S. gallinarum* in the ticks *Ornithodorus moubata* and *Argas persicus* respectively, the essential phases were that the spirochaetes broke up into granules in the various organs of the tick, such as the Malpighian tubules and gonads. Marchoux and Couvy state that such granules may be found in 'the Malpighian tubules, ova and genital ducts' of normal ticks and other Arachnids. They figure granules, often arranged in clumps, in these cells.

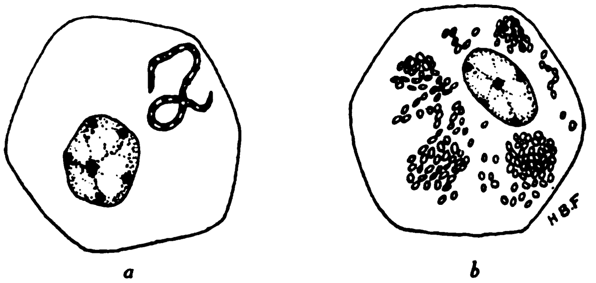
However, all granule clumps seen in Arachnid cells are not of the same nature, as may be observed in fresh preparations with dark-ground illumination, when differences of refractility are apparent.

The chief points raised by Marchoux and Couvy may be considered and answered:

(1) It was never asserted by Leishman and his followers that granules do not occur in normal tick cells. Their presence was quite well known to the present writer from dissection of control ticks (*Ornithodorus moubata* and *Argas persicus*), kept under similar conditions, and from examination of *O. savignyi* and *Ixodes ricinus*, though in many cases the granules were not so numerous therein as Marchoux' figures suggest to have been the case in his ticks.

(2) It was never asserted that all intracellular granules in infected ticks are spirochaetal in origin.

(3) Looking at the figures of Marchoux and Couvy, and from knowledge of my own dissections, it may be stated, in general terms, that the granules—whether they be discrete or in clumps—in normal tick cells are much more uniformly arranged than are spirochaetal granules. (See text-fig., *b*.)



Spirochaeta duttoni **developing in** ***Ornithodorus moubata.***—Mononuclear cells of the tick containing (*a*) Spirochaete dividing into coccoid bodies. (*b*) Similar tick-cell containing granules (coccoid bodies or spores). Such mononuclear cells occur in various organs of ticks and in developing Malpighian tubules. Similar structures have been found in large mononuclear (endothelial-like) cells found in bronchial expectoration infected with ***S. bronchialis.***

Since first writing this paragraph I have made a comparison of the published photomicrographs of Leishman (1909) and Balfour (1911, *Fourth Wellcome Report*, Plate IV, fig. 1), and my conclusion is sustained.

(4) It is possible to see spirochaetes breaking up into granules in infected tick cells, and some of the granules may still be spirally arranged. (See text-fig., p. 475.)

In this connection it is interesting to note that R. M. Carter (1907) described and figured specimens of *S. duttoni* forming chromatin granules within themselves inside ova of *O. moubata.*

(5) The spirochaetal granules themselves are capable of division, forming relatively large clumps or aggregates, different from those figured by Marchoux and Couvy.

The granules found inside spherules, formed in the manner figured by the authors (1913) on p. 453, are not Leishman granules.

(6) According to Marchoux, the spirochaetes within an infected tick retain their spirochaetal facies, becoming at times so attenuated that they may cease to be visible, some having been observed already on the limits of visibility. If they are invisible, how is a determination of their facies possible?

Emphasis is laid on the use of gentian violet as a stain for detecting thin spirochaetes. The value of that stain in researches on spirochaetes was known to me years ago, and was published in my papers of 1907-08.

(7) The French authors state (p. 460) that 'if Argas in which spirochaetes cannot be seen in the coelomic fluid be made to feed on a healthy fowl, we record that some hours after the feed fine spirilla are present.'

Whence come these fine spirochaetes? Apparently, according to Marchoux, from the invisible, ultra-microscopic forms—surely a *petitio principii.* According to the Leishman school they would arise from the granules produced by the multiple fission of pre-existing spirochaetes.

(8) With regard to the metamorphosis of thin slender spirochaetes into larger, thicker ones, by nutrition influencing growth—on which much stress is laid—this is a matter of common knowledge. Morphological variation in spirochaetes, 'attributable to the processes of growth and division,' was described by me in 1909

for *S. balbianii*, and by Fantham and Porter (1909) for *S. duttoni* and *S. recurrentis*.

That thin Herpetomonads (e.g., *H. jaculum*) grow into stout granular forms when fed on blood was shown by Porter in 1909. Robertson (1912) explains polymorphism in trypanosomes (e.g., *T. gambiense*) on somewhat similar lines, namely, as 'due to growth and division phenomena.'

Marchoux and Couvy's elaborate experiments to show that thick spirochaetes result from thin ones by appropriate feeding is merely proving the obvious, and has no direct bearing on the Leishman granule.

(9) The statement by the authors on p. 461 that 'the English authors have certainly injected spirilla, at all events fine spirilla, at the same time as the granules,' is a mere assertion without either proof or justification, as Marchoux and Couvy did not examine the material used by 'the English authors.'

(10) Apparently our critics consider that, although the chromatin granules are regularly arranged inside the periplast, the 'fragmentation' of spirochaetes in the alimentary tract of a tick is merely due to digestion and degeneration. They quote the occurrences observed in blood containing spirochaetes kept at different temperatures, or taken from ticks at various periods after incubation at 28° C. But no one would consider the 'flagellation' of male malarial crescents in blood cooled on a slide as a phenomenon of degeneration; on the contrary, it is merely the same as occurs naturally in the digestive tube of the mosquito. Similarly, the fragmentation of spirochaetes in the digestive tract of a cold-blooded invertebrate like Argas or Ornithodorus is also natural, and not degenerative. It is not accurate to say that such granules generally lose their staining power.

Unfortunately, structures not understood at first have often been styled 'degenerate' in the past. The stout forms of polymorphic trypanosomes in the blood, for example, when a few chromatoid granules were noticeable in them, were formerly said to be degenerative. Now it is known that such forms are important and represent the adult type.

As regards the failure to infect vertebrates by the injection of coccoid bodies—on which some stress has been laid—that, unfortunately, is sometimes, though not always, the case. Perhaps, as Hindle (1912, p. 474) remarks, there is some undetermined factor (? coxal fluid) connected with the development of coccoid bodies in such cases.

Having come to the pathetic conclusion (p. 468) that 'it is easier to state what the Leishman granules are not than what they really are,' after considering the possibilities of them being bacteria, mitochondria or pre-secretory grains, and admitting that when kept at 37° C. the Leishman bodies elongate and become bacilloid and 'vibrioniennes,' the authors leave us without any positive guidance. Destruction only, not construction!

Blanc's researches were discussed in my paper of 1911. He doubts the connection of granules seen in infected *Argas persicus* with the life-cycle of *S. nicollei* (a Tunisian variety of *S. gallinarum*).

Wolbach (1914) sometimes found disintegrating spirochaetes common in the gut cavity of ticks. The parasites were breaking up into chromatin granules. He found 'spirochaetes in the muscular and connective tissue structures of practically every organ of the body.' He did not find spirochaetes in the cells of the Malpighian tubules. The author thinks that his results support the conclusions of Marchoux and Couvy. Such, however, is hardly the case. Personally, I have frequently found spirochaetes in every organ of the body of an infected tick, especially if the tick had previously been kept for a short time at 30° C. to 35° C. Many investigators seem to overlook the importance of recording the temperature or other climatic conditions under which the ticks, dissected or otherwise used by them, were previously kept.

Thus, taking some of the conclusions set forth by Wolbach, it has not been denied that spirochaetes migrate through all the organs and tissues of the tick. In explaining one figure the author states that the tissue 'is in relation to a Malpighian tube. Note the absence of spirochaetes or granules in the epithelium.' The conditions are stated to be forty-seven days after feeding the tick. Might not the granules have changed into spirochaetes meanwhile, and left the actual Malpighian epithelial cells? This aspect of the question seems to be quite overlooked by the author and others of the same school of thought.

Apparently Wolbach is anxious to uphold the views of Dutton and Todd (1907) and Breinl (1907)—investigators who were among the earliest to record granule formation in spirochaetes—by using differential terms such as 'infective granules,' 'large granules,' and 'comma bodies,' but without clearly stating the distinctions between them. He finds large spirochaetal granules in connective tissues of infected ticks, and gives interesting results of the examination of sections of tissues of such ticks. Exception, however, would be taken by cytologists to his statement that 'tissues may remain in the fixative [sublimate-alcohol] for several weeks without harm.'

V. FURTHER RECENT RESEARCHES SHOWING THE RELATION OF GRANULES IN THE LIFE-CYCLE OF SPIROCHAETES

Noguchi, in his epoch-marking series of papers on the cultivation of Spirochaetes and Treponemata, published in 1911 and 1912, records interesting observations on the granules of *S. phagedenis*. He writes (1912) that 'the granules . . . liberated [from the spirochaete] seem to remain alive, and at certain periods develop into spiral forms.' Again, in his paper on the cultivation of *Treponema pallidum* (1911), Noguchi records, in the explanation of one of his figures, that 'it is not rare to find a round body connected with one or two young pallida, as though the latter were just sprouting from the former. . . . The size of this spore-like round body is variable.' However, it should be noted that in these various papers Noguchi records several kinds of granules that may be found in cultures of spirochaetes, some of which, apparently, are not related to the progressive life-cycle of the organisms.

There is further evidence to show that spirochaetal granules obtained from organs of infected ticks grow *in vitro* into spirochaetes. Thus, Balfour (1913) writes: 'I *seem* in a few cases to have succeeded in growing spirochaetes *in vitro* from tick eggs containing only granules, so far as could be told.'

Sir William Leishman (1913) repeated many of his former experiments with altered technique, employing dark-ground illumination instead of fixation and staining. He finds that 'the granule-clumps are not all alike, but that some of them show a much higher degree of refractility than others. Continuous observation of these refractile granules, with the microscope in a thermostat, has further shown me, in the case of two ticks, the definite extrusion of small and actively motile spirochaetes from these granule clumps.' Sir Wm. Leishman is still of opinion that the granule clumps described are derived from spirochaetes. He continues: 'After about ten days at a temperature of 28° C. to 30° C., and with a relatively high humidity, some of these clumps give rise to young spirochaetes, which become free, are actively motile, multiply by fission, and may persist in the tissues of the tick for many months.'

The mention of the conditions of temperature and humidity in the foregoing quotation should be carefully noted. Balfour (1913) also mentions the same, and adds 'general climatic conditions.' These factors undoubtedly influence the development of spirochaetes, especially in the invertebrate hosts, as I have repeatedly found in my own researches.

Last year (1913), while working in Khartoum, I made careful studies, using dark-ground illumination, of *S. bronchialis*, *S. gallinarum* (Sudanese variety), and *S. recurrentis* (the variety styled by some *S. berbera*). In each case refractile granules or cross-bars could be seen in the spirochaetes during certain stages of development in the appropriate fluid of the vertebrate host. The thicker forms of *S. bronchialis*, for example, clearly showed this phenomenon. Fixed and stained preparations were confirmatory. Mononuclear cells found in the bronchial expectoration showed within them spirochaetes breaking up into granules and the individual granules resulting therefrom. Some of these granules were separate, some arranged in a snake-like line, others were in clumps. The granules must be the cross-infective stages of *S. bronchialis*, for those (e.g., Chalmers and O'Farrell, J. A. Taylor and myself) who have worked for any length of time with that organism find that the spirochaete phase dies very rapidly outside the human body.

Since writing the foregoing, I have read with great interest two recent papers by Drs. Edmond Sergent and H. Foley on the parasite of North African relapsing fever, named by them *S. berbera* in 1910. It is to these authors working in Algeria that we owe the first demonstration (1908-9) of the transmission of such spirochaetes by lice. In their latest work (June and July, 1914) the authors find that *S. berbera*, which is possibly only a variety of *S. recurrentis*, assumes in the louse a very small form which is as virulent as the spirochaetiform stage. During eight days following a meal of infected blood the body of the louse does not contain any spirochaetes as such, though the spiral organisms reappear later, as first observed by Nicolle, Blaizot and Conseil in 1912 in Tunis. Sergent and Foley also find that a similar minute but infective form occurs in the blood of relapsing fever patients during apyretic intervals when spirochaetes are absent. Apparently their researches are not yet complete, as they do not mention the morphology of this minute, infective form of the spirochaete in lice. Very probably it will be found to be of the nature of a Leishman granule or coccoid body.

Nicolle and Blanc (June, 1914) have been working at the same problem—*S. berbera* in lice—in Tunis. They conclude that the causal agents of relapsing fever are virulent or infective in the louse just before they reappear as spirochaetes. They think that there is an invisible stage in the evolution of the spirochaetes in the louse. But the authors do not mention granules; have they considered such a stage? Why do they postulate an invisible stage when they apparently have not examined for a granule stage, which might be overlooked by too great reliance on the use of the 'ultra-microscope.'?

These various results and suggestions must be carefully considered before further unproved generalisations are made.

VI. CONCLUSIONS

In conclusion, the following points, among others, may be reiterated and emphasised:

1. It is generally accepted, at any rate by those who have worked on the organisms, that spirochaetes form granules by multiple fission. The formation of such granules, variously known as Leishman granules, coccoid bodies or spores, has been observed by various workers in molluscan spirochaetes, in blood spirochaetes, and in *Treponema pallidum*, among others. The significance of the granules, whether cyclical or degenerative, only is in dispute.

2. There is danger of generalisation on incomplete evidence in

the case of spirochaetal granules. It has never been asserted by any of the investigators who have continued and confirmed Leishman's work that *all* granules seen in the various tissues of infected ticks (*Ornithodorus moubata* and *Argas persicus*), especially those of the gonads and Malpighian tubules, were of spirochaetal origin. Nor was it ever asserted by Leishman and his supporters that other granules, for example, of a possible mitochondrial or secretory nature, could not occur in tick cells.

3. The present writer in his experiments examined control ticks as far as possible, as well as other ticks, such as *Ornithodorus savignyi* and *Ixodes ricinus*, which were not infected with spirochaetes. The granular structures figured by Marchoux and Couvy (1913) were sometimes seen in the various tissues of the ticks, but often they were not so marked as represented in the figures of those authors.

4. The necessity for detailing the climatic conditions, especially of temperature and humidity, under which experimental ticks whether infective or non-infective were kept, cannot be too strongly urged.

5. Morphological variation in spirochaetes, due to nutrition affecting growth and division, is well known, and was discussed by the present writer in 1909. The value of gentian violet as a stain in the investigation of spirochaetes was recorded by the writer in 1907 and 1908. These two points, on which Marchoux and Couvy lay stress, are not new.

6. Marchoux and Couvy's postulate of 'invisibly' thin or ultra-microscopic spirochaetes is distinctly weak, and seriously vitiates their contention that spirochaetes remain as such throughout their existence in the tick, and that they must occur as spirochaetes in inoculation material.

7. Small spirochaetes have been seen to form and emerge from clusters of refractile spirochaetal granules in fresh infected tick material when examined under dark-ground illumination. Such spirochaetes may invade any organ of the tick and remain therein for long periods.

8. There is evidence to show that spirochaetal granules obtained from the organs of infected ticks grow *in vitro* into spirochaetes.

9. Detailed and prolonged studies of *Spirochaeta bronchialis* have been made in Khartoum by the writer. It was found that the spirochaete formed granules (coccoid bodies or spores) and that these were the cross-infective stages of the organism. The spirochaetiform phase of *S. bronchialis* dies rapidly outside the human host.

10. Recent important researches by Sergent and Foley (1914) have shown that the causal agent of North African relapsing fever, besides its spirochaetiform stage, possesses a very small and equally virulent form which it assumes during apyrexial periods in man, and during a period following an infecting meal in the louse. Very probably such a minute form is of a granular or coccoid nature. Nicolle and Blanc (1914) conclude that the organisms are infective in the louse just before they reappear as spirochaetes.

REFERENCES

Further references will be found at the ends of some of the papers cited.

Balfour, A. (1911). The rôle of the Infective Granule in certain Protozoal Diseases. Brit. Med. Journ., Nov. 11, No. 2,654, pp. 1268-1269. See also Journ. Trop. Med. and Hyg., XIV, pp. 113-114 and pp. 263-264; and Fourth Report Wellcome Laboratories, pp. 93-96.

——— (1912). The Life-cycle of *Spirochaeta gallinarum*. Parasitology, V, pp. 122-126.

——— (1913-14). Notes on the Life-cycle of the Sudan Fowl Spirochaete. Trans. XVII Internat. Congress of Med., London, 1913. Sect. XXI, Trop. Med and Hyg., Pt. II, pp. 275-278.

Blanc, G. R. (1911). Les Spirochètes : Leur évolution chez les Ixodidae. 129 pp. Two plates. Thèse de la Faculté de Médecine. Paris, Jouve et Cie.

Bosanquet, W. C. (1911). Brief Notes on the Structure and Development of *Spirochaeta anodontae*. Quart. Journ. Microsc. Sci., LVI, pp. 387-393. One plate.

Breinl, A. (1907). The Morphology and Life-history of *Spirochaeta duttoni*. Annals Trop. Med. and Parasitol., I, pp. 435-438. One plate.

Carter, R. M. (1907). The Presence of *Spirochaeta duttoni* in the Ova of *Ornithodoros moubata*. Annals Trop. Med. and Parasitology, I, pp. 155-160. One plate.

Chalmers, A. J., and O'Farrell, W. R. (1913). Bronchial Spirochaetosis. Journ. Trop. Med. and Hyg., XVI, pp. 329-334.

Dutton, J. E., and Todd, J. L. (1907). A note on the Morphology of *Spirochaeta duttoni*. Lancet, Nov. 30, 1907, pp. 1523-1525.

Fantham, H. B. (1907). *Spirochaeta* (*Trypanosoma*) *balbianii*, its movements, structure and affinities; and on the Occurrence of *Spirochaeta anodontae* in the British Mussel, *Anodonta cygnea*. (Prelim. Communic.) Ann. and Mag. Nat. Hist., Ser. 7, Vol. XIX, pp. 493-501.

——— (1908). *Spirochaeta* (*Trypanosoma*) *balbianii* (Certes) and *Spirochaeta anodontae* (Keysselitz): Their movements, structure and affinities. Quart. Journ. Microsc. Sci., LII, pp. 1-73. Three plates.

——— (1909). The Spirochaetes found in the Crystalline Style of *Tapes aureus*: A Study in morphological variation. Parasitology, II, pp. 392-408. One plate.

——— (1911). Some Researches on the Life-cycle of Spirochaetes. Annals Trop. Med. and Parasitology, V, pp. 479-496.

Fantham, H. B., and Porter, A. (1909). The Modes of Division of *Spirochaeta recurrentis* and *S. duttoni* as observed in the Living Organisms. Proc. Roy. Soc., B, LXXXI, pp. 500-505.

Gross, J. (1912). Ueber Systematik, Struktur und Fortpflanzung der Spironemacea. Centralbl. f. Bakt., I, Orig., LXV, pp. 83-98.

Henry, H. (1913). A consideration of the Infective Granule in the Life-History of Protist Organisms. Journ. Path. and Bact., XVIII, pp. 250-258.

Hindle, E. (1911). The Transmission of *Spirochaeta duttoni*. Parasitology, IV, pp. 133-149.

——— (1912). On the Life-cycle of *Spirochaeta gallinarum*. Parasitology, IV, pp. 463-477.

Hoffman (1912). Zur Stellung der Spirochäten im System. Centralbl. f. Bakt. Abt. 1. Orig. LXVI, pp. 520-523.

Leishman, W. B. (1909). Preliminary Note on Experiments in connection with the Transmission of Tick Fever. Journ. Roy. Army Med. Corps, XII, pp. 123-135.

——— (1910). An Address on the Mechanism of Infection in Tick Fever, and on Hereditary Transmission of *Spirochaeta duttoni* in the Tick. Lancet, CLXXVIII, Jan. 1, pp. 11-14. Also Trans. Soc. Trop. Med. and Hyg., III, pp. 77-95.

——— (1913-14). Relapsing Fevers. Trans. XVII Internat. Congress of Med., London, 1913. Sect. XXI, Trop. Med. and Hyg., Pt. II, p. 282.

Marchoux, E., and Couvy, L. (1912). Argas et Spirilles (Spirochètes). Bull. Soc. Path. Exotique, V, pp. 63-68 and pp. 796-798.

——— ——— (1913). Argas et Spirochètes. Annales Inst. Pasteur, XXVII, pp. 450-480 and pp. 620-643.

Nicolle, C., et Blanc, G. (1914). Les Spirilles de la fièvre récurrente sont-ils virulents aux phases successives de leur évolution chez le pou ? Démonstration de leur virulence à un stade invisible. C. R. Acad. Sci., Paris, t. 158, pp. 1815-1817.

Noguchi, H. (1911). A Method for the Pure Cultivation of Pathogenic *Treponema pallidum* (*Spirochaeta pallida*). Journ. Exptl. Med., XIV, pp. 99-108. Five plates.

——— (1912). Pure cultivation of *Spirochaeta phagedenis* (new species), a spiral organism found in phagedenic lesions on human external genitalia. Journ. Exptl. Med., XVI, pp. 261-268. Two plates.

Porter, A. (1909). Some Observations on Living Spirochaetes from Lamellibranchs. Arch. Zool. Expér. et Gén., 5e Ser., III, pp. 1-26.

——— (1909). The Life-cycle of *Herpetomonas jaculum* (Léger), parasitic in the Alimentary Tract of *Nepa cinerea*. Parasitology, II, pp. 367-391. One plate.

Robertson, M. (1912). Notes on the Polymorphism of *Trypanosoma gambiense* in the blood, and its Relation to the Exogenous Cycle in *Glossina palpalis*. Proc. Roy. Soc., B, LXXXV, pp. 527-539.

Sergent, Ed., et Foley, H. (1914). Des périodes de latence du Spirille chez le malade atteint de fièvre récurrente. C. R. Acad. Sci., Paris, t. 158, pp. 1926-1928.

——— ——— (1914). De la période de latence du Spirille chez le Pou infecté de fièvre récurrente. C. R. Acad. Sci., Paris, t. 159, pp. 119-122.

Wolbach, S. B. (1914). The Distribution and Morphology of *Spirochaeta duttoni* and *Spirochaeta kochi* in experimentally infected Ticks (*Ornitbodorus moubata*). Journ. Med. Research, XXX, pp. 37-48. Three plates.

Zopf, W. (1882). Zur Morphologie der Spaltpflanzen (Spaltpilze und Spaltalgen). 74 pp. and 7 plates. Leipzig, von Veit & Co.

Zuelzer, M. (1911). Ueber *Spirochaeta plicatilis* Ehrbg. und deren Verwandtschaftsbeziehungen. Arch. f. Protistenkunde, XXIV, pp. 1-59. Four plates.

ON A CASE OF *NASO-ORAL LEISHMANIASIS* (CORRESPONDING TO THE DESCRIPTION OF *ESPUNDIA*); AND ON A CASE OF *ORIENTAL SORE*, BOTH ORIGINATING IN THE ANGLO-EGYPTIAN SUDAN

BY

J. B. CHRISTOPHERSON, M.D. (CANTAB.), F.R.C.P. (LOND.), F.R.C.S. (ENG.)

DIRECTOR OF KHARTOUM AND OMDURMAN CIVIL HOSPITALS

(*Received for publication August* 11, 1914)

PLATES XXVI, XXVII

INTRODUCTION

Two cases of leishmaniasis from the Anglo-Egyptian Sudan are described in this paper; one was naso-oral in character, the other was a case of Oriental Sore.

The two cases are worthy of notice in several respects.

The form of leishmaniasis known as Espundia* has not yet been reported in this part of the world, nor, as far as I know, anywhere else than in South America. A second point of interest is that it undoubtedly originated in the district of the Sudan where most of the cases of kala azar come from and seem to originate, namely, Sennaar on the Blue Nile—and where there are undoubtedly cases of Oriental (Aleppo or Bagdad) Sore.

---

* Espundia. See page 1556 Castellani and Chalmers, Manual of Tropical Medicine, 2nd Edition (1913).

The above case undoubtedly corresponds to the description of Espundia, but I should probably not have so described it had not Dr. Chalmers pointed out the resemblance of the photograph to the picture in the above work.

The naso-oral case was as follows:

Faragalla Rish (Plate XXVI, fig. 1), a Bergawy, or partly Arab partly negroid race, male, aged about 40. He was born in Western Sudan, and being a slave had been brought to Omdurman during the Mahdi's time, that is, about the year 1883. He had afterwards settled up the Blue Nile, and for 'years' has been living at a place called Wad el Abbas near to Sennaar Town.

He had been in Khartoum twelve days when he came to the hospital in March, 1914. The sore, he stated, commenced on the upper lip about two years previously. It dried up during the summer according to him, and when I saw him the lip was almost healed so far as the ulcer was concerned; but, as will be seen from the photograph (fig. 2), the whole upper lip was much swollen and protruding, and looked as if it were the seat of a chronic inflammatory process. The bridge of the nose was fallen in. On the floor of the nose there was a good deal of ulceration extending as far back as could be seen. The cartilaginous septum was almost entirely destroyed (figs. 3, 5). The ulcerated surface was irregular in shape and extended deeply, the carious bone being felt with a probe on the floor of the ulcer.

I could not see whether the mucous membrane of the turbinated bones was involved; as far as I could tell it was not.

There was some ulceration on the inner part of the alae nasi. All the ulcers were crusted over by a thick yellow coagulated secretion or scab, which was easily lifted off in large masses by means of forceps, and which consisted of nasal secretion and muco-purulent secretion from the ulcerated surface.

The inside of the mouth was also involved in the following way:

There were two somewhat deep ulcers with overhanging edges on the under aspect of the upper lip close to the frenum, where the lip joins the gum (see fig. 4). The mucous membrane of the gums of the upper front teeth, as far laterally as the canine, looked swollen, spongy, and papillomatous, presenting a velvety appearance but not ulcerated (see fig. 4). In front there was a distinct line marking the separation of the healthy from the infected gum. Immediately behind, and, in fact, surrounding the incisor teeth, there was an area of somewhat deep ulceration which extended between the teeth. This ulcerated area was narrow but deep, and extended as far laterally as the back of the canines (see Plate XXVII, fig. 6).

Inside the mouth the part of the upper jaw affected corresponded to the area of distribution of the greater palatine vessels, and the ulceration possibly made its way along the lymphatics accompanying the vessels passing through the foramen incisivum from the floor of the nose to the mouth. The external ulcer on the lip when I saw it was an irregular shallow healing ulcer on a somewhat hard base, and about the size of a thumb-nail, in fact almost healed over.

Scrapings of the ulcers on the outside of the lip, the inside of the nose, and from the ulcerated area behind the teeth, and also of the ulcers on the inner aspect of the upper lip, all contained Leishman-Donovan bodies (fig. 7) in scanty numbers, and were found and stained by my pathological assistant, Mr. Newlove.

The history was that the disease commenced on the upper lip below the nose. The lip is well now except for thickening. There were no enlarged lymphatic glands, the liver and spleen were not enlarged, the temperature was normal, the blood count was normal, and there was nothing else to note about the man except that he was poorly nourished.

With regard to the appearance of the Leishman-Donovan bodies, some of them, though not all, would bear out the description of those found in cases of Espundia, namely, that the large nucleus is somewhat flatter and more spread out and extended. Others corresponded to the type of Leishman-Donovan bodies usually seen in tropical sore.

The cultures which were made failed to grow, although tried on media growing the kala-azar organism. Owing to a misunderstanding the man was discharged from hospital. (He has since been readmitted.)

ORIENTAL SORE

The second case is one of true Oriental Sore, and it also originated in the Egyptian Sudan, but in Khartoum North, and not in the same district as the so-called Espundia case (Sennaar).

An Egyptian clerk named Faluny Luka, in the Khartoum North Zaptia, aged 22, came to the Khartoum Civil Hospital on

March 19th, 1914. He had an ulcerated swelling over the metacarpo-phalangeal joint of the little finger of the right hand (fig. 8), and a festering sore on the skin over the lower end of the ulna bone on the left wrist. He arrived in Khartoum North from Egypt in December, 1912. No sore was noticed anywhere until July, 1913. There was no enlargement of liver or spleen, the temperature was normal, and he was apparently otherwise healthy. The appearance of the sore on the right hand is well shown in the photograph (fig. 8), and needs little description.

The part was swollen and looked inflamed, there were a good many chaps or cracks corresponding to the wrinkles in the skin running round the sore part. These were covered with a yellow crust; on the summit of the swelling was a festering sore which was not painful. There was also a smaller sore on the left wrist—a circular, festering, superficial crusted sore covered with a yellow scab. Both contained the Leishman-Donovan bodies.

The young man was not in poor health but was well nourished and sturdy-looking. The sores, he said, before he came to hospital, got bigger fairly rapidly, but he could not say how long they took to reach their maximum size. He had no idea how he became infected, and knew no one, either in his own family or outside it, with similar sores. No kind of animal was kept in the house. He used to wear a small ring on the affected little finger. The sores on both hands got well within a month under treatment.

DISCUSSION

The above two cases illustrate two clinical forms of leishmaniasis, apart from kala-azar, found in the Sudan. The one had all the characteristics of Aleppo boil (Bagdad Sore), and the other all the clinical characteristics of the naso-oral leishmaniasis known in Peru under the name of Espundia, and described by Splendore as not so rare in Brazil as is supposed. In the Kala-Azar Report (Sudan Government) for the year October 1912—October 1913 (a copy of which Col. Bray, R.A.M.C., kindly sent for my perusal), Bimbashi Marshall, alluding to the close association between human and canine leishmaniasis and kala-azar and Oriental Sore, states that 'curiously enough in the area of the Sudan infected by kala-azar neither canine leishmaniasis nor Oriental Sore have been found.' At the same time he says that he is not satisfied that they are not present.

The first of the cases reported in this paper undoubtedly originated in the area of the Sudan most affected with kala-azar. Dr. Chalmers of the Wellcome Research Laboratories has since shown me a preparation made from a case of cutaneous leishmaniasis (Oriental Sore) coming from this district; so that probably there are more cases in the Sudan than is supposed. I think that we may say that Oriental Sore, naso-oral leishmaniasis and kala-azar all exist in the Sudan, and in the same district.

There is an interesting fact (noted by Marshall in the Kala-Azar Report, Sudan, 1912-13) that Martoglio has recorded three cases of Oriental Sore in Abyssinia, and suspects the presence of kala-azar in that country.

A good many of the already reported cases in the Sudan have been of Abyssinian nationality. Though none has been proved exclusively to have received infection in Abyssinia, it is a fact that the two most infected provinces are Sennaar and the Kassala border on the Abyssinian frontier. Sennaar is on the high road, so to speak, between Abyssinia and Khartoum. Incidentally we may be almost certain that kala-azar is present in Abyssinia as well as Oriental Sore.

With regard to the so-called Espundia case, the following summarises what is known of Espundia or naso-oral leishmaniasis.

'Espundia' has been reported up to the present time from nowhere but South America, although it has been known as a clinical entity long before 1911. It was in 1911 definitely associated with the Leishman-Donovan body by Splendore, and also by Carini.

Carini draws attention to the fact that a leishmaniasis of the nose and throat has a characteristic clinical appearance, and that the mucous membrane manifestation of leishmaniasis nearly always occurs in individuals who have had cutaneous ulcers of the same kind in other parts of the body (in this case on the lips). Although in some cases propagation by continuity of skin lesion must be admitted, in other cases the mucous localisation commences first on the palate at the back of the mouth when simple extension could not be admitted. He does not favour a theory that the infection of the mucous membrane is caused by transportation, e.g., by finger-nails.

Dr. Alfonso Splendore (Rome), who has written, from cases in Brazil, as much as anyone on the subject, says 'the disease is much less rare than one would imagine.' In one of his cases the lesion began on the skin of the face, in other cases it began on the mucous membrane of the mouth.

In one case he mentions the primary lesion was on the face; it then appeared on the tonsils, spreading from thence to the adjacent parts of the mouth, and finally appearing in the nasal cavity. He identifies the naso-oral leishmaniasis of Brazil with the so-called 'Espundia' of Peru. He was able to obtain cultures from his cases, the Leishman-Donovan body cultivated differing from that of Aleppo Boil in the length of its flagellum (50 μ). He also maintained, with Laveran and Nattan-Larrier (1912), that it is a distinct variety, and acquiesced in the name of *L. tropica* var. *americana* proposed by Laveran and Nattan-Larrier. His inoculation experiments do not appear to have been successful. He observes that its commencing, as it sometimes does, in the mucous membrane of the mouth supports the old view that leishmaniasis is transmitted by water.

Escomel (1911) appears first to have suggested an analogy between the primary chancre of syphilis and the primary sore of Espundia, which latter, he observes, is usually on the forearm, legs, neck, or chest, followed, it may be after some years, with manifestations on the mucous surfaces, namely, naso-, bucco- and pharyngeal mucosa. In the case recorded by me the period was two years.

How far the analogy with syphilis is fanciful is not for me to say, having only experience of the case I report.

Laveran and Nattan-Larrier, writing on material sent them by Escomel, went a step further and noticed that the Leishman-Donovan bodies found in Espundia possess a special point of peculiarity, namely, that the nucleus is flattened and pressed against the wall of the body. This appeared to them a constant feature in Escomel's specimens and was searched for in vain in the preparations of *Leishmania donovani* or *Leishmania tropica* in their possession. But, as they say, their observations were insufficient to enable them to draw any conclusion.

Wenyon found among the Leishmania of a Bagdad button bodies possessing flattened nuclei pressed against the wall, and he also obtained, in the case of South American Leishmania, cultures quite similar to those of *Leishmania tropica.* His culture and inoculation experiments led him to the conclusion that there is no difference between Leishmania of Oriental Sore and Leishmania of Espundia. However, Laveran and Nattan-Larrier observe that, even when no appreciable morphological differences could be seen between the American Leishmania and *Leishmania tropica*, it would be possible to distinguish those parasites just as one distinguishes *Leishmania donovani* and *Leishmania tropica*, although these two Leishmania present the greatest possible degree of resemblance from the point of view of morphology. They conclude: 'The Leishmania of Buba and Espundia appear to us to constitute, if not a new species, at least a variety of *Leishmania tropica* to which we propose to give the name *Leishmania tropica* var. *americana.*'

Prof. Laveran thinks that the names Buba and Espundia should disappear, and that it is reasonable to give the name of American Leishmaniasis thereto. But this on the face of it suggests that the disease is peculiar to America.

The disease is probably existent in other parts of the world and awaits discovery. It would seem that the name naso-oral leishmaniasis is preferable, as it identifies it clinically with the nose and mouth where it invariably is situated, without suggesting more than that of which we are absolutely certain.

I publish these two cases together because, although clinically the case of naso-oral leishmaniasis seems undoubtedly to correspond to what is known as Espundia in South America, the further question as to whether Espundia is to be differentiated from cutaneous leishmaniasis as a distinct entity again arises. I think it necessary to draw attention to the fact that typical cutaneous leishmaniasis (Oriental Sore) does originate in the Sudan,* occurring in the same area from which most of the kala-azar cases come. This case of 'Espundia' came from the area from which both kala azar and also cutaneous leishmaniasis (tropical sore) come. Dr. Chalmers, who has seen the case, examined the slides and has been good enough to have made some experiments in cultivations and inoculations which have not so far been successful,* but which will be repeated again as soon as possible He writes me a short note as follows:—

---

* Besides my own case I may mention another:—Dr. Chalmers showed me, before I left Khartoum on leave in April, 1914, the slide of a case of Tropical Sore which he had made from a patient coming from the Kala Azar district of the Blue Nile.

'KHARTOUM, 19.7.14

Herewith some remarks and extracts:—

Diagnosis:—The points of peculiarity in this case of Leishmaniasis are:—

(1) It is a local infection of the skin, the nose and the mouth, and not a general infection of the body as evinced by the blood count, the absence of an enlarged spleen and the absence of fever.

(2) It begins with an ulcer on the upper lip which is said to have lasted two years and which was nearly healed when the patient was first seen. This ulcer showed 'Leishmania bodies.'

(3) Long after the commencement of the primary infection the nose became infected and later the mouth. In both Leishmania bodies can be found but are very rare.

All these points agree with the clinical symptoms of 'Espundia,' the question now arises, is 'Espundia' to be differentiated from 'Cutaneous Leishmaniasis'?

The only points of difference which can be pressed are:—

(1) Difference in parasite, nucleus, ease of cultivation, flagellum, scarcity.

(2) Clinical differences.

(3) Geographical distribution, and none of these to my mind are at present conclusive.'

My own opinion is that clinically this case is one of so-called Espundia, but it does not so far throw much light on the further question whether this distinct clinical entity is due to a distinct variety of the Leishman-Donovan body.

The fact that we have twice failed to grow cultures on media that grow easily the kala-azar organism of the same district might possibly be taken as some evidence indicating a distinct variety of the micro-organism, but it must be said that Leishman-Donovan bodies, although demonstrated in the sores of the lip, the nose, and the mouth, were in each case scanty and required much searching for.

---

* I may say that twice cultures have failed to grow on media on which the Kala-Azar (Sudan) organisms are growing.

On the other hand, the case came from a district where both kala-azar and the form of leishmaniasis known as Oriental Sore are endemic.

I hope that culture and inoculation experiments will throw further light on this interesting question.

REFERENCES

de Amicis (1909-10). Report on cases of Buba. Congrès International de Medicine, Budapest (1909), Comptes Rendus Sect. XIII, Dermatologie, 1910, p. 409.

Carini, A. (1911). Leishmaniose de la muqueuse rhino-bucco-pharyngée. Bull. Soc. Path. exotique, IV, p. 289.

Castellani and Chalmers (1913). Manual of Tropical Medicine, 2nd edition, p. 1556.

——— references on p. 1576 :—

(*a*) Bueno de Miranda (1910). Arch. de Soc. de Med. de S. Paulo.

(*b*) Breda (1899-1912) numerous papers in Giornale Malattie della Pelle e Annali Medicina Navale.

(*c*) Verrotti (1911) Giorn. Int. Scienze Medich. [Papers which I have been unable to procure (J.B.C.).]

Escomel, E. (1911). Bull. Soc. Path. exotique, IV, p. 489.

Laveran and Nattan-Larrier (1912). Contribution à l'étude de la Espundia. Bull. Soc. Path. exotique, V, pp. 176, 486.

Reports of Kala Azar Commission (Sudan) 1909-1913.

Splendore, A. (1911). Arch. f. Schiffs- u. Tropen-Hygiene, XV, p. 105.

——— (1912). Bull. Soc. Path. exotique, V, p. 411.

Wenyon, C. M. (1912). Journ. Trop. Med. and Hyg., Vol. XV, July 1, p. 193.

EXPLANATION OF PLATES

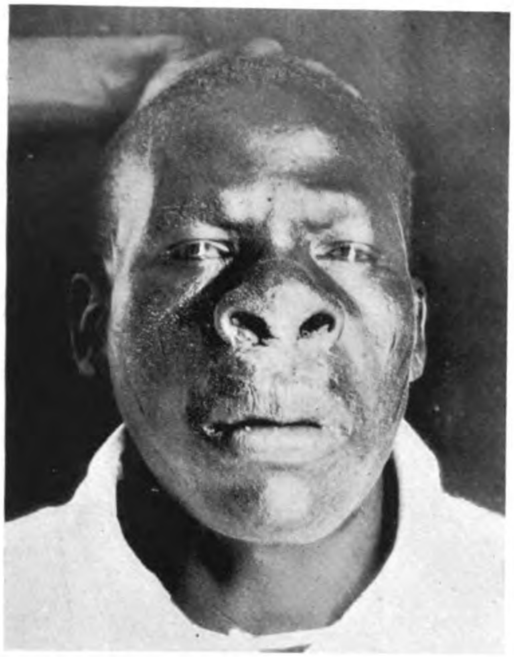
PLATE XXVI

Fig. 1. Espundia—shows ulceration on upper lip with much thickening; depressed bridge of nose.

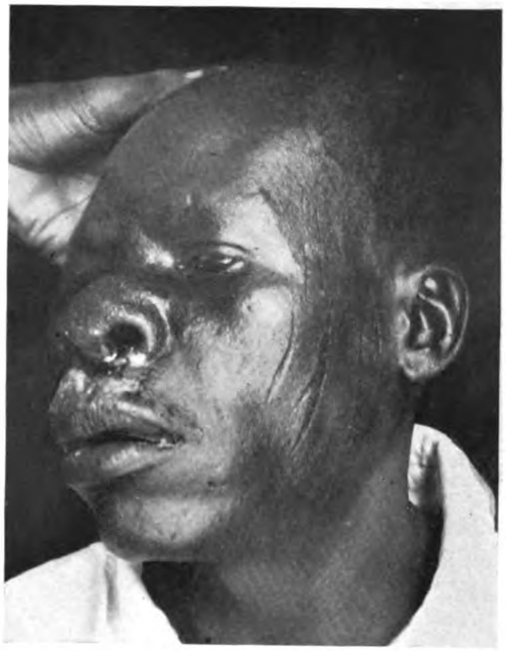
Fig. 2. Shows side view of same.

Fig. 3. Shows healed ulcer on upper lip and ulceration inside nares.

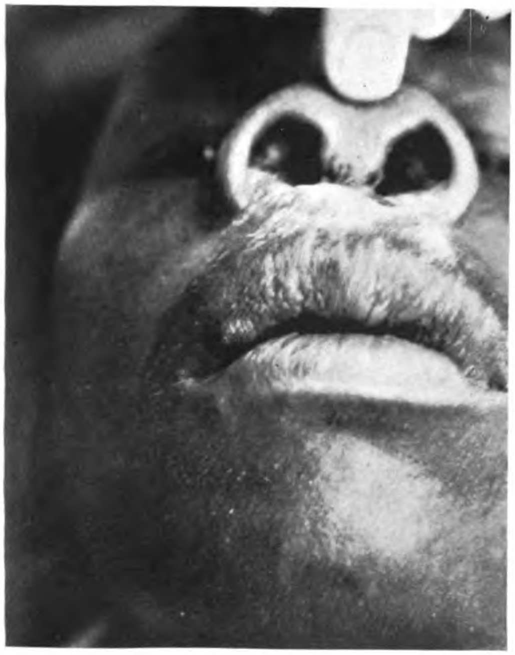
Fig. 4. Irregular ulcer with overhanging edges near frenum of upper lip, from which Leishman-Donovan bodies were obtained. Also velvety roughened condition of mucous membrane of upper jaw from canine tooth of one side to canine tooth of other side. There seemed to be a distinct line on either side marking off healthy mucous membrane from that affected by the disease.



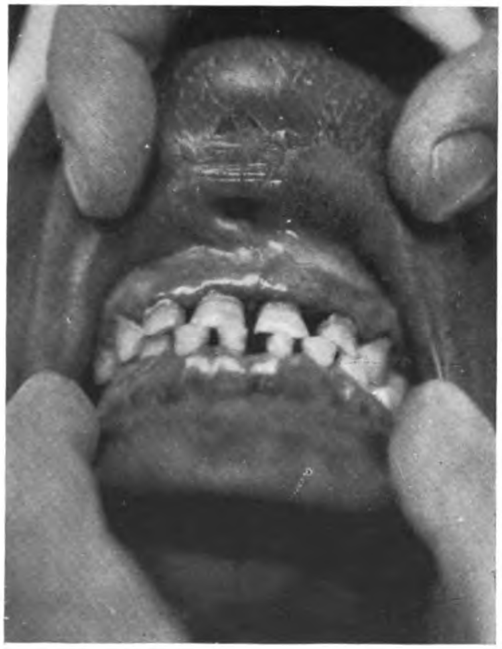
I



2



3



4

C. Tinling & Co, Ltd., Imp.

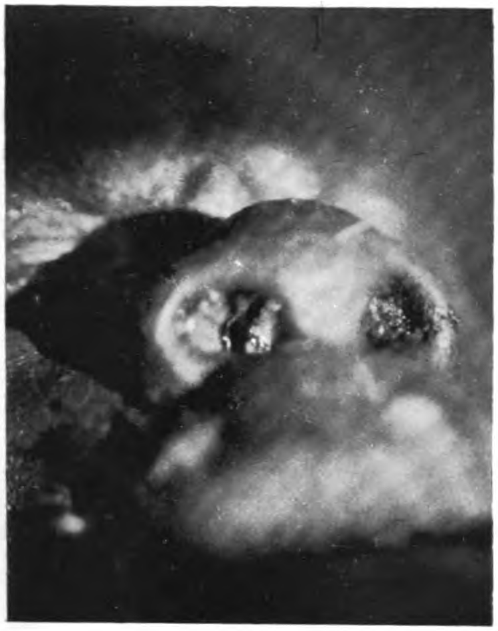
PLATE XXVII

Fig. 5. Espundia—showing ulceration within alae nasi and perforation of septum nasi. Photograph taken with a bright light reflected up left nostril.

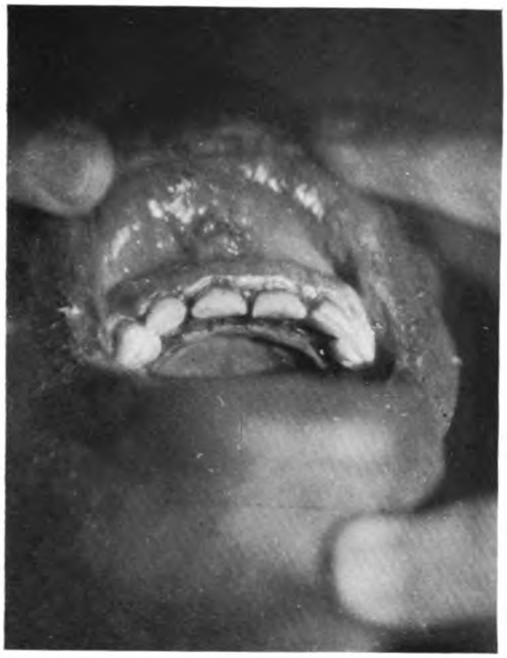
Fig. 6. Espundia case showing ulceration immediately behind upper incisors, also ulcer of frenum.

Fig. 7. Micro-photographs of Leishman-Donovan bodies from Espundia case. × 1400.

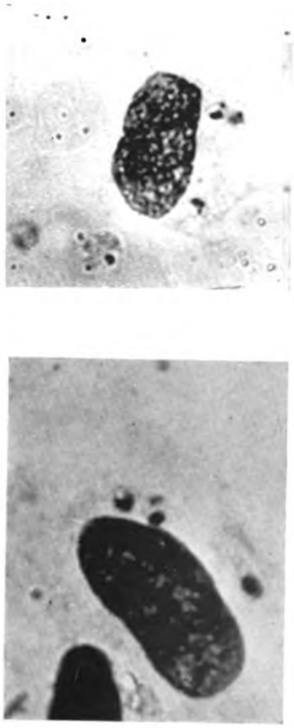
Fig. 8. Oriental Sore on both hands of Faluny Luka.



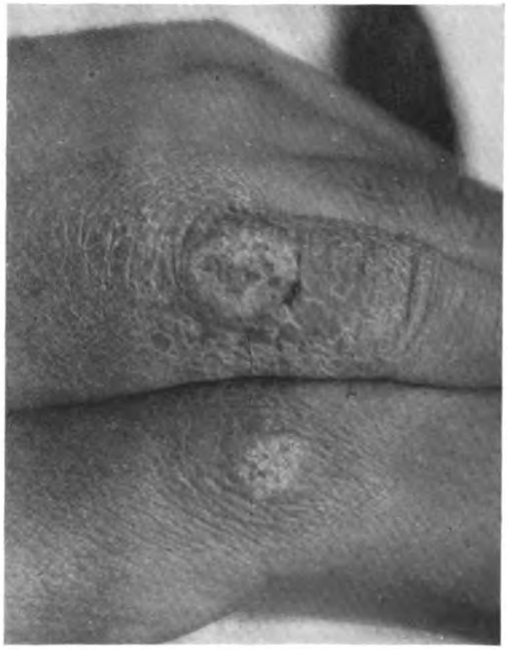
5



6



7



8

C. Tinling & Co., Ltd., Imp.

QUELQUES OBSERVATIONS PRÉLIMINAIRES SUR LA MORPHOLOGIE ET LA BIOLOGIE DE LA LARVE, DE LA NYMPHE ET DE L'IMAGE DE L' *AUCHMEROMYIA LUTEOLA*, FABR.

PAR

LE DR. J. SCHWETZ

(*Received for publication February*, 1914)

INTRODUCTION

Arrivé à Kabinda pour deux-trois mois, j'ai voulu profiter de ce séjour pour faire entr'autre une collection de 'larves des planchers du Congo' (Congo floor Maggot) dont j'avais promis l'envoi à plusieurs personnes. Tout en mettant les larves apportées dans de l'alcool j'en ai mis également un certain nombre dans deux flacons avec un peu de sable.

Quelques jours plus tard j'ai trouvé dans mes flacons des pupes, et une quinzaine de jours après j'ai aperçu dans les flacons plusieurs mouches. Cela m'a donné l'idée d'en élever moi-même un certain nombre et de profiter de cet élevage pour faire quelques observations. J'ai donc commencé à m'en occuper assez systématiquement.

En deux mois j'ai élevé une cinquantaine de mouches. Il me reste encore beaucoup de nymphes non écloses et beaucoup de larves vivantes pas encore empupées, mais je suis forcé d'interrompre mes observations devant partir pour un long voyage. C'est pour cette même raison que je dois pour le moment renoncer à quelques expériences que j'aurais voulu faire avec la larve.

Je recommencerai mes observations plus tard dès que j'aurai l'occasion de rester dans un poste au moins pendant deux mois.

J'ai déjà cependant pu faire quelques constatations morphologiques et surtout biologiques qui n'ont pas encore été signalées par les auteurs et je crois utile de les publier,—quitte à les compléter plus tard,—sous forme de notes préliminaires.

QUELQUES REMARQUES MORPHOLOGIQUES

La morphologie de la mouche adulte est bien décrite par Manson dans son manuel de Pathologie exotique. Voici la description donnée par cet auteur.

'La mouche mesure de 10 à 12 millimètres de long et son corps est plutôt trapu. Sa couleur générale est chamois, mais de nombreux petits poils noirs lui donnent un aspect enfumé. La tête est large, avec des yeux bien séparés chez les deux sexes. Le thorax présente deux bandes longitudinales foncées, mal définies, qui n'atteignent pas son bord postérieur. L'abdomen diffère dans les deux sexes, le second segment chez la femelle étant deux fois plus long que chez le mâle. Le premier segment a une étroite bande noirâtre sur son bord postérieur chez les deux sexes; le second segment chez le mâle est marqué par une bande plus large qui s'amincit en avant, le long, de la ligne médiane jusqu'à la base du segment. Chez la femelle la bande est noirâtre et si large qu'elle occupe presque tout le segment.

'Le troisième segment est presque entièrement noir chez les deux sexes. Le quatrième est foncé à la base et plus clair postérieurement. Les pattes ont la même couleur que le reste du corps. Le premier article du torse est noir de jais et se détache d'une manière très apparente sur le pulvillus blanc-crème. Les ailes ont une couleur brun-fumée et des nervures visibles.'

Je crois devoir préciser quelques points.

1. L'*Auchmeromyia luteola* est dessinée chez Manson (et d'après lui, également dans d'autres manuels) sans tâches distinctes sur le thorax, ce qui est inexact. La mouche a quatre tâches brun foncées sous forme de deux paires de bandes longitudinales sur le proto-thorax et le mesothorax. Les deux bandes du proto-thorax occupent toute la longueur de ce segment et sont un peu plus larges et plus foncées et ont des limites plus nettes que les deux bandes du mesothorax. Ces dernières qui sont en somme la continuation des deux premières, n'atteignent pas le bord postérieur du segment. En d'autres termes, le thorax présente deux bandes longitudinales foncées n'atteignant pas son bord postérieur et nettement coupées par la ligne interthoracique.

2. La femelle est plus grande en général et spécialement plus

longue que le mâle. La femelle mesure 13 mm.; le mâle 11 mm. Sur 133 Auchméromyies j'ai trouvé 4 exemplaires notablement plus petits, mais la proportion reste à peu près la même: femelles, 10 mm.; mâles, 9 mm. Cette différence de grandeur est si notable qu'on peut presque déterminer le sexe de la mouche avant d'examiner les détails caractéristiques, mais cette différence est surtout frappante quand on a l'occasion de voir deux auchméromyies *in coitu*. Cette différence de grandeur provient de ce que le deuxième segment abdominal est beaucoup plus long chez la femelle—presque le double: 3 mm. au lieu de 2 mm.—que chez le mâle.

Ce deuxième segment présente un dessin très différent, et typique, dans les deux sexes.

(*a*) *Chez le mâle*. Sur le bord postérieur du deuxième segment abdominal se trouve une bande étroite noire (mais plus large que celle, une vraie ligne, du bord postérieur du premier segment abdominal). Perpendiculairement à cette bande, sur la ligne médiane, se trouve une autre bande noire, longitudinale donc, qui va en s'amincissant vers le bord antérieur du deuxième segment qu'elle n'atteint pas. On peut comparer ce dessin à une ancre dont les branches seraient droites. Dans certains cas la bande longitudinale est très courte, n'est qu'ébauchée, et alors elle a la forme d'un petit triangle dont la base est posée sur la bande noire transversale. A noter que cette bande est très noire et se détache d'une manière apparente sur le troisième segment de couleur plutôt cendrée.

(*b*) *Chez la femelle* presque tout le deuxième segment, sauf le tiers ou le quart antérieur est noir. Le bord antérieur de cette tâche noire est irrégulier et peu net, mais il présente en général deux petites échancrures, une de chaque côté de la ligne médiane.

OBSERVATIONS BIOLOGIQUES

Les mâles sont beaucoup plus fréquents, presque le double, que les femelles. Sur 133 auchméromyies, dont une cinquantaine élevées par moi-même, j'ai trouvé 84 *mâle*s et 49 *femelles*.

Au commencement de septembre j'ai mis dans deux flacons remplis de sable jusqu'à la moitié vingt grosses larves gorgées de sang qui venaient de m'être apportées d'un petit village voisin. Le 15 septembre en examinant les flacons, je n'ai plus trouvé qu'une seule larve: toutes étaient transformées en pupe. Vers la fin de septembre les pupes ont commencé à éclore, et au commencement d'octobre tout était presque fini. Les deux derniers pupes ont éclos avant le 15 octobre.

Les larves qu'on m'apportaient n'étaient bien entendu pas uniformes. Il y en avait des grandes et des petites, des blanches (à jeun), des rouges (fraichement gorgées de sang) et des noires (contenant du sang dégéré). Et je noterai dès à présent un fait curieux quoique très simple.

Toutes mes larves et mouches provenaient d'un petit village voisin. Pour m'en procurer je payais aux enfants de ce village un prix fixe pour chaque larve et chaque mouche. Eh, bien, au commencement on m'apportait, et en très grand nombre, presque exclusivement de grandes larves rouges ou noires. Peu à peu les larves moyennes et blanches ont commencé à apparaître et, plus tard, à prédominer. Vers le troisième mois de cette 'exploitation' je n'obtenais que de rares larves très petites et blanches que les enfants devaient encore très soigneusement chercher. Ce fait s'explique d'autant plus facilement que les enfants collectionnaient en même temps très soigneusement les adultes dont le 'prix' était plus élevé que celui des larves.

Déjà à priori il n'était pas difficile de conclure que les grandes larves rouges s'empuperaient plus facilement et plus vite que les petites larves blanches. Il était aussi à prévoir que les toutes petites blanches enfermées dans les flacons, c'est à dire privée de nourriture, ne s'empuperaient pas du tout et finiraient par mourir après un jeun trop prolongé. Il restait donc à confirmer par la pratique ces quelques déductions théoriques. Une autre question se posait encore: quelle larve s'empuperait-elle plus vite? Une grande blanche (adulte à jeun) ou une petite rouge (jeune nourrie)?

J'ai donc divisé les larves en trois catégories, d'après leur grandeur:—

(1) Grandes: 10 mm. et davantage.
(2) Petites: environ 3 mm.
(3) Moyennes: 5-7 mm.

J'ai en outre divisé les larves en deux catégories suivant leur couleur, c'est à dire suivant le laps de temps écoulé depuis le dernier repas:—

(1) *Rouges* ou *noires*: dernier repas tout récent ou plus ou moins récent.

(2) *Blanches*: jeun prolongé ou même absolu.

Voici quelques résultats:

(1) 3 *grandes larves rouges*:
23. IX.—Mises en flacon.
30. IX.—3 pupes.
12. X.—2 images et 1 pupe.
13. X.—3 images.

(2) 3 *petites larves rouges*:
23. IX.—Mises en flacon.

Au commencement novembre 2 larves sont mortes et désséchées. La troisième vit encore le 27 novembre, après un jeun de plus de deux mois. Elle a augmenté de volume et est devenue une 'moyenne.'

(3) 3 *larves moyennes blanches.*
23. IX.—Mises en flacon.
30. IX.—2 pupes et 1 larve.
11. X.—1 image, 1 pupe et 1 larve.
27. XI.— idem.

(4) 4 *larves moyennes et* 1 *petite*: *toutes noires.*
7. X.—Mises en flacon.
14. X.—2 pupes, 2 larves légèrement empupées à une extrèmité, 1 larve.
27. X.—1 image, 2 pupes, 1 larve vivante, 1 larve morte.
31. X.—2 images, 2 pupes, 1 larve désséchée.
27. XI.— idem.

(5) *Une grande larve et deux petites*: *toutes rouges et très mobiles.*
9. X.—Mises en flacon.
14. X.—Même état. Les deux petites sont devenues grandes.
20. X.—2 pupes et 1 larve.
30. X.—1 image et 2 pupes dont 1 aplatie et désséchée.

(6) 3 *larves moyennes et* 1 *petite—rouges.*

15. X.—Mises en flacon.
20. X—Même état.
30. X—Même état.
10. XI.—2 pupes et 2 larves.
27. XI.—2 pupes aplaties et désséchées, 1 petite pupe de 6 mm. (l'ordinaire mesure 10 mm.), 1 larve bien mobile.

(7) 1 *grande larve,* 7 *moyennes et* 3 *petites—toutes blanches.*

16. X.—Mises en flacon.
20. X.—Même état.
27. XI.—3 pupes désséchées, 1 pupe aplatie, 2 larves immobiles, 5 *larves très mobiles.* (N.B.—La plus petite d'entr 'elles mesure 5 mm., les autres—7-10 mm.).

(8) 4 *petites larves rouges très mobiles et* 1 *grande blanche peu mobile.*

17. X.—Mises en flacon.
20. X.—Même état.
30. X.—2 pupes, 3 larves.
27. XI.—1 pupe, 3 pupes désséchées et aplaties, 1 larve morte.

(9) 2 *grandes larves blanches,* 2 *grandes rouges et* 3 *moyennes un peu noires.*

18. X.—Mises en flacon.
20. X—Même état.
30. X.—2 pupes (les 2 grandes rouges), 5 larves.
8. XI.—2 images, 1 pupe, 2 larves, 2 larves un peu empupées.
10. XI.—3 images, 2 larves, 2 larves un peu empupées.
27. XI.—3 images, 1 larve vivante, 1 larve désséchée, 2 petites pupes désséchées.

(10) 11 *grandes et* 5 *petites larves: toutes rouges et mobiles.*

20. X.—Mises en flacon.
27. X.—Toutes les larves sont devenues blanches.
30. X.—4 pupes.
5. XI.—8 pupes.

9. XI.—2 images.
11. XI.—3 images.
12. XI.—4 images.
14. XI.—5 images.
20. XI.—7 images.
27. XI.—7 images, 3 grandes larves vivantes, 4 larves en partie empupées et mortes, 2 petites pupes désséchées.

N.B.—Dans ce flacon le sable contenait beaucoup de minuscules pierres ce qui a probablement empéché le développement des larves un peu trop serrées par dessus le marché à cause de leur grand nombre.

(11). 5 *grandes larves rouges*, 3 *petites—rouges*, 4 *grandes blanches et* 3 *petites blanches.*

30. X.—Mises en flacon.
10. XI.—4 pupes.
20. XI.—3 images.
27. XI.—3 images, 2 pupes aplaties, 1 petite pupe, 2 larves fraîchement empupées (peu foncées et encore molles), 7 larves mobiles (3 grandes et 4 moyennes).

On voit donc que l'élevage des auchméromyies, sans qu'on nourrisse les larves au laboratoire, donne beaucoup de succès, pour ainsi dire 100 %, quand on choisit de grandes larves rouges, c'est à dire arrivées à maturité et bien nourries. Le succès est moins grand quand on emploie de 'grandes blanches.' On a encore beaucoup moins de succès lorsqu'on a recours aux petites larves même si ces dernières sont rouges. Le résultat est tout à fait négatif quand on a affaire aux 'petites blanches.'

La durée de la période larvaire de l'auchméromyie dépend donc uniquement des conditions alimentaires, de la fréquence des repas, et varie d'un minimum 'X' jusqu'à plusieurs mois. La larve peut en effet supporter un jeun extrèmement long. Dans mon observation No. 2, une petite larve rouge a supporté un jeun de plus de deux mois (depuis le 23 IX jusqu'au 27 XI), et qui sait combien de temps elle continuera encore cette existence avant de mourir ou de se . . . nymphoser, peut-être. Même phénomène dans l'observation No. 3 ('blanche moyenne').

Les observations sur les larves nées au laboratoire n'avanceraient donc pas la résolution exacte de cette question (de la durée de la période larvaire). J'ai d'ailleurs eu l'occasion d'avoir à deux reprises des larves nées chez moi, mais, comme on va le voir, le résultat a été peu brillant.

(1) Le 31. X, au matin on m'apporte un tube contenant 4 auchméromyies qui viennent d'être capturées: 2 mâles et 2 femelles. J'aperçois dans le tube quelques oeufs et je laisse continuer la ponte. Le soir j'enlève les oeufs et en compte 60. J'en mets 10 dans une boite en verre contenant un peu de sable humide. Le 6. XI, je ne vois encore aucune larve. Le sable étant toujours humide je l'expose au soleil, et déjà 30 minutes après je trouve 3 larves très mobiles et légèrement plus longues que les oeufs.

Le 16. XI, deux larves sont encore très mobiles, mais elles ne grandissent pas, c'est à dire n'atteignent pas encore 2 mm. de long. La troisième larve est morte (évidemment d'inanition). Je trouve dans le sable deux membranes vides, ressemblant à première vue aux oeufs. Les autres 7 oeufs sont désséchés et déjà un peu déformés. Le 18. XI, les deux larves restantes sont également mortes.

Deux larves ont donc vécu sans la moindre nourriture depuis la naissance pendant 12 jours, et la troisième 10 jours.

(2) Le 16. XI, au matin on m'apporte dans un tube 2 auchméromyies: 1 mâle et 1 femelle. Pendant que le tube se trouve sur ma table de travail la femelle commence à pondre. Le lendemain matin, le 17, je compte 25 oeufs. La femelle est déjà morte. Je suis empêché de m'en occuper, et quand j'ouvre le tube le lendemain matin, le 18, je trouve dans un petit creux du bouchon une petite larve éclose donc en moins de 48 heures. Elle a un peu plus d'un mm. et est très mobile. Elle est de couleur blanc-mat sauf l'extrémité antérieur effilée qui est noire. Je la mets dans du sable sec et la surveille tous les jours. Le 22. XI, 11 jours donc après l'éclosion et malgré le jeun complet, la petite larve est très alerte. Je la mesure et trouve 1½ mm. de long. Pendant la mensuration la petite bestiole s'est accrochée à mon doigt. Par une secousse instinctive de la main la larve est tombée par terre et je ne l'ai plus retrouvée malgré les longues recherches.

La durée de la période pupale a été dans mes observations d'une dizaine de jours (8-15). Quand la pupe reste trop longtemps (plus de 20 jours, par exemple) sans éclore il est probable que c'est une pupe 'ratée' et qu'elle finira par se désśécher. Cela arrive surtout chez les pupes provenant des larves pas assez mûres, soit trop petites, soit pas assez nourries, ce qui revient d'ailleurs au même. Ces pupes 'avortons' se reconnaissent par leur petite taille.

La période nymphale, dans les premiers jours du moins, est une période très critique. N'ayant pas besoin de nourriture et étant immobile la jeune nymphe de l'auchméromyie est cependant très sensible à toute insulte extérieure, au plus léger traumatisme. Ainsi, je ne suis jamais arrivé à obtenir une image des pupes apportées. J'ai plusieurs fois trouvé des nymphes au lieu des larves apportées, c'est à dire que la nymphose avait eu lieu avant que j'ai eu le temps de mettre les larves dans les flacons. Connaissant dans ces cas exactement 1 heure de nymphose je tenais beaucoup à ces exemplaires pour pouvoir constater exactement la durée de la période nymphale, mais toutes ces nymphes étaient régulièrement trouvées aplaties et désséchées au bout de quelques jours, n'ayant pas pu supporter même un léger maniement. J'ai eu enfin beaucoup de nymphes 'perdues' (aplaties et désséchées) dans les flacons ou le sable contenait quelques petits cailloux. En penchant le petit flacon pour voir ce qui se passait dedans, c'est à dire en déplaçant le sable, je mortyrisais ainsi les pupes délicates. Les larves par contre, grâce probablement à leur élasticité, supportaient parfaitement bien ces secousses.

Les larves d'auchméromyies sont de vraies fouisseuses, in vitro du moins. Jetées dans un récipient contenant du sable les larves y disparaissent immédiatement et traversent en quelques instants une couche sablonneuse de 4-5 cm. Une fois dans le sable les larves se tiennent relativement tranquilles le jour, mais leurs moeurs changent soudainement à l'arrivée de la nuit.

Cette mobilité nocturne m'a frappé dès le commencement de mes observations.

Dans l'après-midi j'ai examiné mes quelques flacons où, à première vue, sans remuer le sable en tournant les récipients, je ne voyais pas une seule larve. Mais en cherchent un objet, le soir, entre les flacons, j'ai aperçu dans ces derniers un grand remue-ménage: toutes les larves se promenaient sur la surface du sable avec une très grande agilité, en se heurtant, en grimpant les unes sur les autres et en essayant de se redresser.

On sait que, dans la nature, les larves d'auchméromyies mordent et sucent le sang spécialement la nuit. 'Elles se nourissent presqu' exclusivement la nuit,' dit Manson. 'La larve se gorge de sang la nuit,' dit Brumpt. Roubaud dit que ce réveil nocturne est un fait d'accoutumance coincidant avec le période du sommeil chez l'hôte. En se basant sur certaines expériences in vitro, le même auteur conclue que les larves d'auchméromyies sont douées d'une irritabilité thermique du 'thermotropisme' en vertu duquel 'ces parasites dispersés dans le sol sont guidés vers leur hôte et parviennent à son contact.'

Ma simple observation citée plus haut infirme cette belle théorie de Roubaud.

En effet, mes larves se trouvant dans des flacons bouchés et placés sur une table n'étaient pas plus 'irritées' la nuit que le jour. L'obscurité n'était pour rien non plus dans cette agilité nocturne des larves, ces dernières ayant continués à s'agiter même quand je plaçais les flacons près d'une grande lampe. Enfin, l'accoutumance *individuelle* n'y était pour rien non plus puisque j'ai pu observer la vivacité spéciale nocturne également chez les quelques larves qui étaient nées chez moi, 'en captivité,' et qui n'avaient jamais eu l'occasion de sucer le sang la nuit ni le jour.

Il faudrait donc admettre que la larve d'auchméromyie est nocturnu par *sa nature* comme, par exemple, la filaire nocturne. Mais si je dis : 'il faudrait' admettre, et non pas 'il faut' admettre, c'est parce que dans la nature la larve ne semble pas être nocturne du tout. . . .

En effet, j'ai interrogé à ce sujet beaucoup d'indigènes qui m'ont déclaré que ce 'ver' piquait parfaitement bien le jour si on se couche dans une hutte par terre, sur une natte ou sur une couchette très basse. La 'larve des planchers' se nourrirait donc *presqu'* exclusivement la nuit parce que c'est *presqu'* exclusivement la nuit que l'on dort.

Mais alors pourquoi est-ce que, in vitro, la larve se remue tant la nuit et non pas le jour ?

Il y a donc encore des lacunes dans nos connaissances des moeurs des larves des auchméromyies.

J'espère pouvoir plus tard recommencer et continuer mes observations sur ces larves haematophages dont le rôle pathogène n'est peut-être pas aussi nul qu'on le croit.

Kabinda, décembre, 1913.

LITTÉRATURE

MANSON. Maladie des pays chauds.

BRUMPT. Précis de parasitologie.

ROUBAUD. Etudes biologiques sur les Auchméromyies. Bulletin de la Société de Pathologie exotique, 1913, No. 2.

RODHAIN ET BEQUAERT. Nouvelles observations sur l'*Auchmeromyia luteola*, Fabr. Revue Zoologique africaine, Vol. II, Fasc. 2-15, février, 1913.

MCCONNEL. Some observations on the larva of *Auchmeromyia luteola*, F. Bulletin of Entomological Research, May, 1913.

THE RELATION OF BILE PIGMENTS TO HAEMOGLOBIN

BY

J. O. WAKELIN BARRATT

AND

WARRINGTON YORKE

(*Received for publication* 4 *June*, 1914)

It has been shown* that haemoglobin is passed through the kidneys into the urine within about ten minutes following the intravenous injection of rabbits with a solution of homologous haemoglobin in physiological saline. If, however, a cannula be inserted into the urinary bladder and the amount of haemoglobinuria estimated at regular intervals it is found that the total quantity of haemoglobin excreted by the kidneys does not as a rule exceed one-fifth of the amount injected, and although the haemoglobinaemia resulting from the intravenous injection has almost entirely disappeared at the end of six hours, there is no evidence available to enable us to state in what way the haemoglobin is removed from the circulation.

For many years it has been held that the bile pigments are closely related to haemoglobin, and are formed as a result of blood destruction which is supposed to be constantly occurring in the normal animal. The work of Tarchanoff† affords support to this hypothesis. This author injected haemoglobin solutions intravenously into dogs, and found that there resulted a great increase in the amount of bile pigment passed.

The experiments described in this paper were devised with the object of further examining this question, and deciding, if possible, whether intravenous injection of homologous haemoglobin in rabbits resulted in the secretion of a bile relatively rich in pigment, and also whether there was any increase in the volume of bile passed and consequently in the total amount of pigment.

---

* Barratt and Yorke. An investigation into the mechanism of Production of Blackwater. Annals of Trop. Med. and Parasitology, 1909, Vol. 3, No. 5, p. 1.

Yorke and Nauss. The Mechanism of Production of Suppression of Urine in Blackwater Fever. Annals of Trop. Med. and Parasitology, 1911, Vol. 5, No. 2, p. 287.

† Tarchanoff. Ueber die Bildung von Gallen pigment aus Blutfarbstoff, 1874. Pflügers Archiv., B. IX, S. 53; Zur Kenntniss der Gallenfarbstoffbildung, ibid., S. 329.

Technique. The ductus communis choledochus was ligatured and a cannula inserted into the gall bladder of rabbits; the operation, which occupied about twenty minutes, was performed in the following manner. The animal was anaesthetised with ether, and the skin over the lower ribs and upper portion of the abdomen carefully shaved and sterilised. An incision was made commencing a little to the left of the middle line over the xiphoid cartilage and running obliquely downwards and to the right parallel with the lower edge of the ribs and about half an inch below them. The common bile duct was then sought at its entrance into the duodenum, and a ligature passed round it as near as possible to its point of entry into the gut; considerable care was necessary in order to avoid injury to the portal vein, an accident which could only be averted by ligaturing the duct close up to the duodenum, at which point it was most widely separated from the vein. After ligaturing the duct the gall bladder was exposed, and a glass cannula (about two inches long), bent at the middle of its length at an angle of about 120°, was inserted through a small incision and tied in so that the ligature included the wounded surfaces of the gall bladder, by which means haemorrhage was arrested. Any bile which escaped during the procedure was absorbed by cotton wool packs. Great care was necessary during this portion of the operation to avoid damaging the liver, either by the instruments or cannula or by tension on the gall bladder. The wound was closed by sutures and sealed with collodion flexile. By means of a piece of rubber tubing attached to the glass cannula the bile was collected as it escaped.

The amount of bile passed was measured from time to time. The intervals in the earlier experiments were greater and more irregular than was desirable, but in the last six experiments the vessel in which the bile collected as it escaped from the tube was changed every hour and accurate estimations of the quantity of bile passed per hour determined, and the amount per kilogramme of body weight calculated.

The bile of normal rabbits, as it escaped from the freshly opened gall bladder, was observed to be of a light greenish-yellow colour. Later in the control experiments the colour of the bile usually changed to a darker olive green; the bile of some animals contained much more yellow pigment than that of others. No attempt was made to determine the actual amount of pigment contained in the bile; our observations were of a purely comparative nature, the concentration of pigment in later specimens being expressed by a multiple or fraction of that occurring in the first specimens examined, which in these experiments is taken as an arbitrary standard and is expressed as unity. The concentrations of pigment in the various specimens of bile escaping from the cannula were compared by means of a Zeiss comparison spectroscope. Although no definite absorption bands are detectable in rabbits' bile, nevertheless there is a distinct darkening and narrowing of the spectrum, light being cut off at each end. For purposes of comparison, the standard bile was placed in a tube 10 or 20 mm. in height, and the relative amount of pigment in the later specimens determined by ascertaining the height of the column, giving a corresponding narrowing of the spectrum. Towards the end of the experiment, when the volume of bile excreted had fallen to a low level, the colour sometimes changed markedly, and the bile acquired a certain degree of brownish tinge. Such change rendered it difficult, or even impossible, to estimate accurately the amount of pigment contained in specimens of bile exhibiting the brownish tinge in terms of the amount of pigment contained in the standard samples, consequently estimations made in such cases are only approximate. Ordinarily, however, determinations were made without any difficulty.

The haemoglobin solution used in these experiments was prepared in the following manner. Rabbits were bled to death from the carotid artery, the blood being collected in a vessel containing about 20 c.c. of a solution of 1 % sodium citrate and ·9 % sodium chloride. The red blood cells were separated by centrifugalisation and washed with normal salt solution. After the third washing as much as possible of the supernatant salt solution was pipetted off, and rather more than an equal volume of distilled water was added to the deposit of red blood cells: the mixture was stirred thoroughly with a glass rod and allowed to stand in an ice chest for half to one hour. Solid sodium chloride was then added in amount sufficient to render the volume of distilled water added isotonic for rabbits' erythrocytes. On the addition of the solid sodium chloride a light red precipitate formed, but this for the most part disappeared on stirring. The red cell stromata were then deposited by centrifugalisation, and the clear supernatant solution of haemoglobin removed by a pipette. The concentration of the haemoglobin solution was determined by means of a haemoglobinometer reading, the absolute values of the scale of the instrument having been ascertained in terms of the red cells (in the moist condition) of one of us taken as a standard.

Solutions of haemoglobin prepared in this manner were injected into the marginal vein of the ear of the experimental rabbits; in a certain number of cases a corresponding quantity of physiological salt solution was injected instead of the haemoglobin solution: the latter animals served as controls.

The experiments can be divided into two series, an earlier series in which examinations of the bile were made at more or less irregular intervals, and a later series in which the bile was measured and the relative concentration of pigment it contained estimated at the end of every hour. This laborious procedure was undertaken in order to obtain more precise information regarding variations in the amount of bile passed and in the relative concentration of pigment contained. The results obtained from each series of experiments are similar, but the various points are more clearly brought out in the tables and charts constructed from data obtained by hourly observation in the later experiments, comprising Rabbits 5, 6, 13, 14, 15 and 16. Two of these (Rabbits 5 and 6) served as controls, 12 c.c. of physiological salt solution per kilogramme of body weight being injected, whilst in the other four animals a solution of rabbit's haemoglobin was introduced into the circulation. Two of these four animals (Rabbits 14 and 15) died with violent convulsions in less than three hours following the injection of haemoglobin solution, the exact cause of death in these cases) is unknown; the subject has been discussed in previous papers,* whilst the other two (Rabbits 13 and 16) survived and appeared but little the worse from the injection. Details of these experiments are given in Charts 1 to 6. Each chart is composed of three curves; the first, indicated by the dotted line, represents the amount, in cubic centimetres, of bile passed per hour per kilogramme of body weight of the animal; the second, indicated by the interrupted line, represents the relative concentration of bile pigment, the first specimens passed before the injection being taken as a standard and referred to as unity, whilst the third, indicated by the continuous line, represents the relative amount of bile pigment passed per hour which is ascertained by multiplying the amount passed per hour by the relative concentration of pigment.

---

* Barratt and Yorke. Ueber Hämoglobinämie. Zeitschr. für Immunitätsf. u. experiment. Therapie, 1912, B. 12.

The Production of General Symptoms in Haemoglobinaemia. Brit. Med. Journal, Jan. 31 1914.

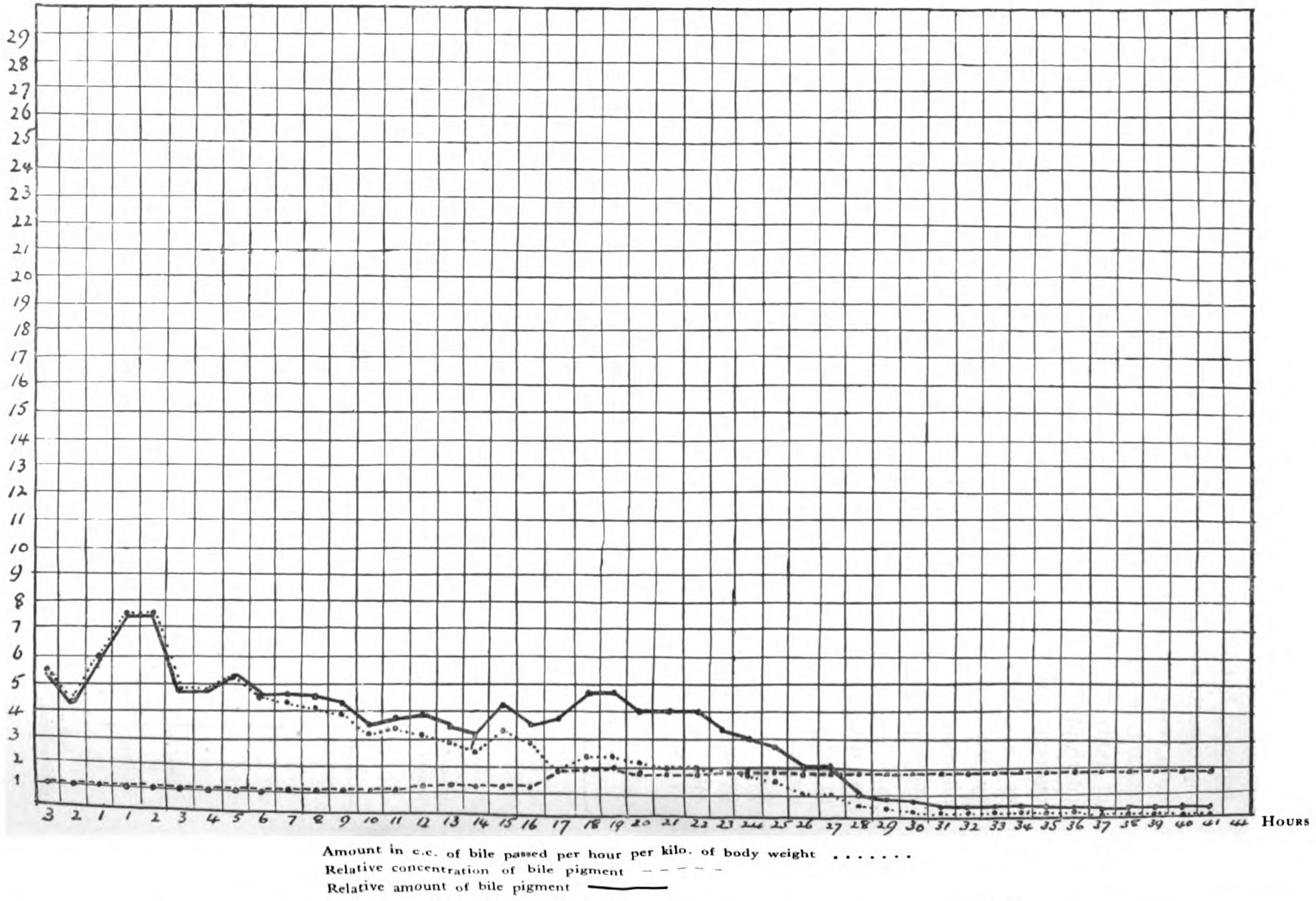
On comparing these tables and charts several points of interest are at once apparent. In every instance there is a definite increase in the volume of bile passed noticeable two to four hours after the commencement of the experiments: possibly this is associated with the recovery of the animal from the anaesthetic, but it is also probably due at least in part to the introduction of salt solution into the circulation. The total output of bile during the first 24 hours of the experiments is given in the following Table. It varied in

Table.—Giving the amount of bile excreted per kilogramme of body weight per hour by the various experimental rabbits.

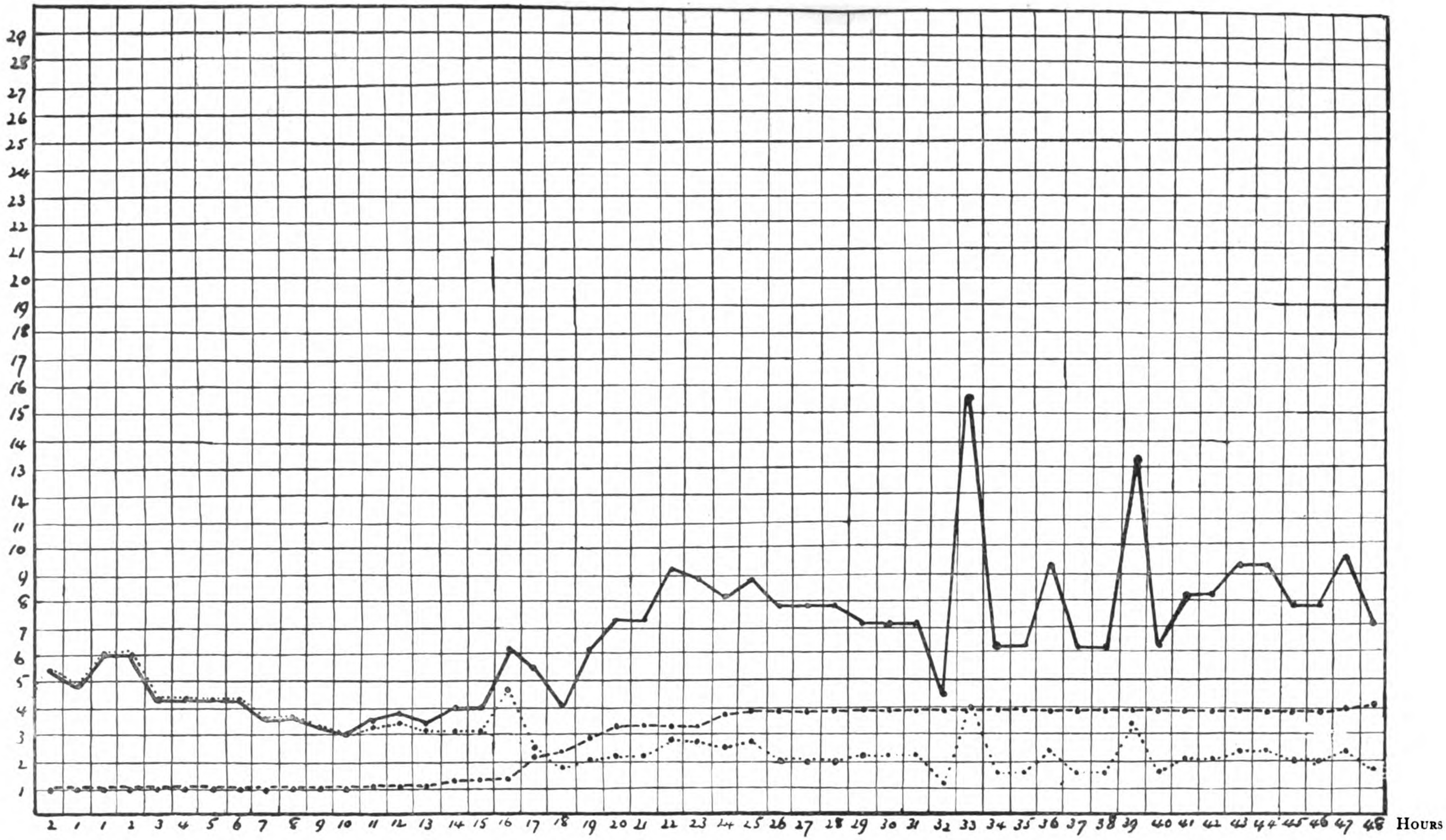
No. of Experiment	Weight in grammes	Amount of bile per kilo. of body weight passed during first twenty-four hours after operation of ligaturing common bile duct and inserting cannula into gall bladder	Nature of intravenous injection	Length of life of animal after operation in hours	Remarks
1	1700	16·75	No injection	78	Animal killed
2	2000	15·0	,,	51	
3	1770	21·0	,,	60	
4	1650	40·0	15 c.c. of ·9% NaCl solution per kilo.	72	
5	1580	91·7	12 c.c. ,, ,,	45	,,
6	1400	87·4	12 c.c. ,, ,,	112	,,
9	1140	84·4	35 c.c. of 50% Hb. solution per kilo.	60	
10	1540	38·0	16 c.c. ,, ,,	28	
11	1800	51·7	16·6 c.c. ,, ,,	140	,,
12	1700	34·5	18 c.c. ,, ,,	36	
13	1500	60·2	3·3 c.c. ,, ,,	42	
16	1500	81·7	14 c.c. ,, ,,	3 weeks	

N.B.—Rabbits 7, 8, 14, and 15 died in less than twenty-four hours after injection of haemoglobin solution.

GRAPH I.—Giving the result of hourly examinations of the bile, as regards volume and concentration of pigment of Rabbit 5 which received intravenously 12 g. per kilo. of ·9 per cent. NaCl solution.



Graph II.—Giving the results of hourly examinations of the bile, as regards volume and concentration of pigment of Rabbit 6 which received intravenously 12 g. per kilo. of ·9 per cent. NaCl solution.



Amount in c.c. of bile passed per hour per kilo. of body weight ....

Relative concentration of bile pigment – – – – – –

Relative amount of bile pigment ———

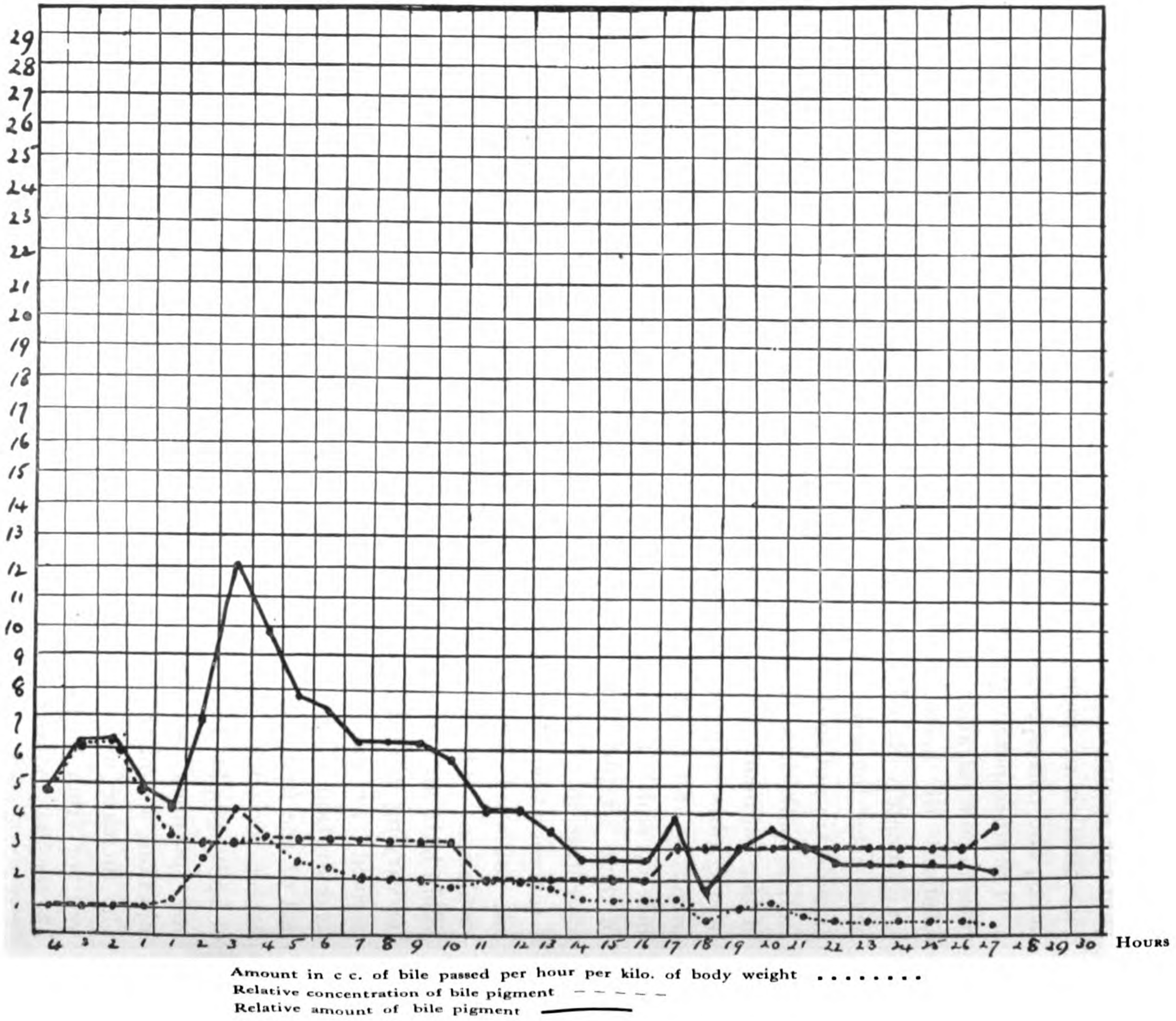
different animals from 15 c.c. to 91·7 c.c. per kilogramme of body weight. If the experiments recorded in this table be divided into the following three groups—1st, those in which there was no injection; 2nd, those in which sodium chloride solution was injected; and 3rd, those in which haemoglobin solution was injected—it is found that the average output of bile during the first 24 hours is in the first group 17·5 c.c., in the second 73 c.c., and in the third 58·5 c.c. These figures show clearly that introduction into the circulation of either sodium chloride solution alone or sodium chloride solution containing haemoglobin causes an increased flow of bile. Another point of interest in this connection is that notwithstanding the increased secretion of bile due to sodium chloride solution, the relative concentration of pigment remains constant. This is clearly brought out in Experiments 5 and 6 (see Charts 1 and 2).

After the initial increase the volume of bile excreted gradually decreased until the death of the animal, which occurred after several hours or was delayed for as long as three weeks. In the latter case the amount of bile passed diminished to about one-half or one-third of its original volume, and then remained more or less constant until the 18th day, when the cannula came out of the gall bladder. Subsequently very little bile escaped from the wound, and the animal died on the 21st day of the experiment. On post-mortem examination a considerable quantity of bile was found in the peritoneal cavity. There were large areas of necrosis in the liver, and the bile ducts were greatly dilated.

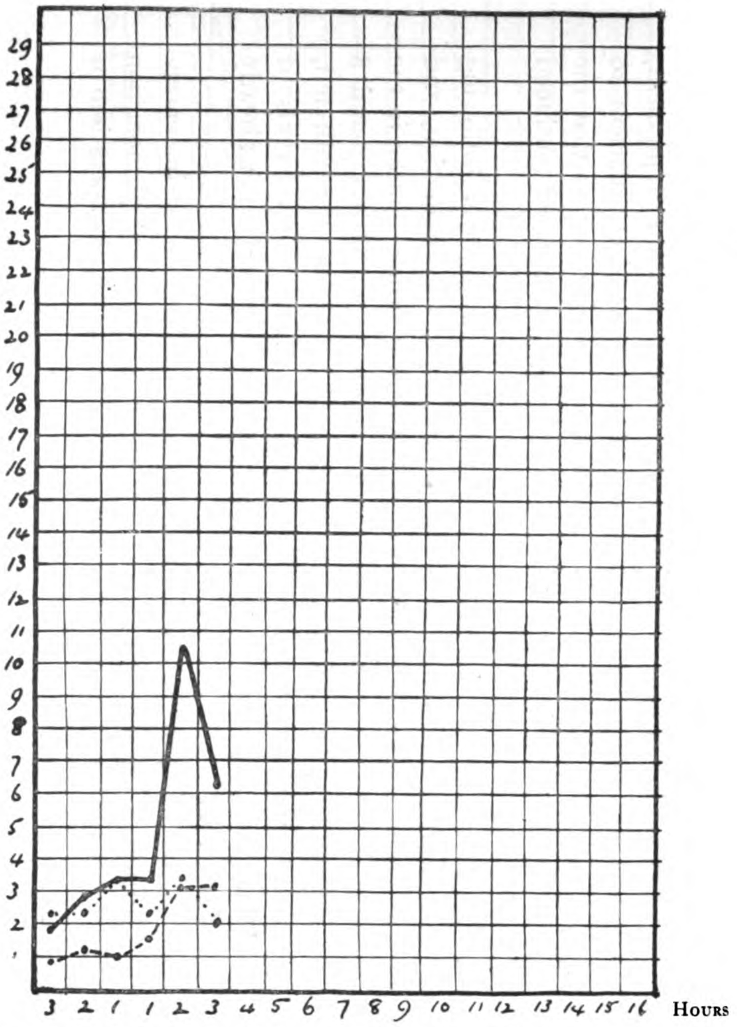
This gradual diminution in the quantity of bile excreted was a constant phenomenon in all our experiments, whether haemoglobin solution had been injected or not, but the curves representing the relative concentration of bile pigment in the control experiments exhibit marked differences from the corresponding curves in the experiments in which haemoglobin solution had been injected. Charts 1 and 2, giving the results of two control experiments (in which ·9 % sodium chloride solution had been injected) show that the curve representing the relative concentration of bile pigment remains at a constant level for about 16 hours after the injection of normal salt solution, or about 18 to 20 hours after the insertion of the cannula into the gall bladder. After this period has elapsed there is a gradual rise in the relative concentration of bile pigment, as is well shown in Chart 2, where it rose to four times the initial concentration and remained constant at this level until the death of the animal. This late increase in the relative concentration of pigment was observed in all our control experiments, and corresponds roughly to the diminution in the bile output.

The corresponding curves given in Charts 3 to 6 depict the variations in concentration of pigment observed in those rabbits in which solutions of haemoglobin have been injected intravascularly. Unfortunately Rabbits 14 and 15 died with convulsions about three hours after the injection, and hence the data obtained from these animals are not so definite or extensive as could be desired. However, in both these the curve representing the relative concentration of bile pigment is seen to rise immediately after the injection of haemoglobin solution. This point is much better brought out in Graphs 3 and 6, which depict the course of events in Rabbits 13 and 16, which lived for considerable periods after the injections. In these graphs the interrupted lines, representing the relative concentration of bile pigment, are seen to rise until they represent a concentration of from four to six times that of the sample of bile collected before the introduction of haemoglobin into the circulation. The increase of concentration of pigment commences within half an hour of the injection of haemoglobin, and continues for varying periods. In the case of Rabbit 13, which had only received 1·6 gm. of haemoglobin per kilo. of body weight, the concentration of bile pigment reached its maximum (four times that of the first specimen) three hours after the injection, whilst in Rabbit 16, which had received 7·0 gm. of haemoglobin per kilo. of body weight, the maximum concentration (5·7 times that of the first specimen) was not observed until the 5th hour after the injection; a similar concentration was also recorded at the 9th hour. The increase in concentration of pigment was maintained fairly well for from 10 to 12 hours, when there was a distinct falling off; the fall, however, never reached unity, that is the concentration of the specimens collected before the introduction of the haemoglobin solution. Sometimes a second rise in concentration corresponding to that observed in the control animals was noticed after 18 to 20 hours, when the volume of the bile output had fallen to a low level.

GRAPH III.—Giving the results of hourly examinations of the bile, as regards volume and concentration of pigment of Rabbit 13 which received intravenously 3·3 g. per kilo. of 50 per cent. haemoglobin solution (1·6 g. Hb.).



Graph IV.—Giving the results of hourly examinations of the bile as regards volume and concentration of pigment of Rabbit 14 which received intravenously 8·2 c.c. per kilo. of 50 per cent. haemoglobin solution (4·1 g. Hb.).

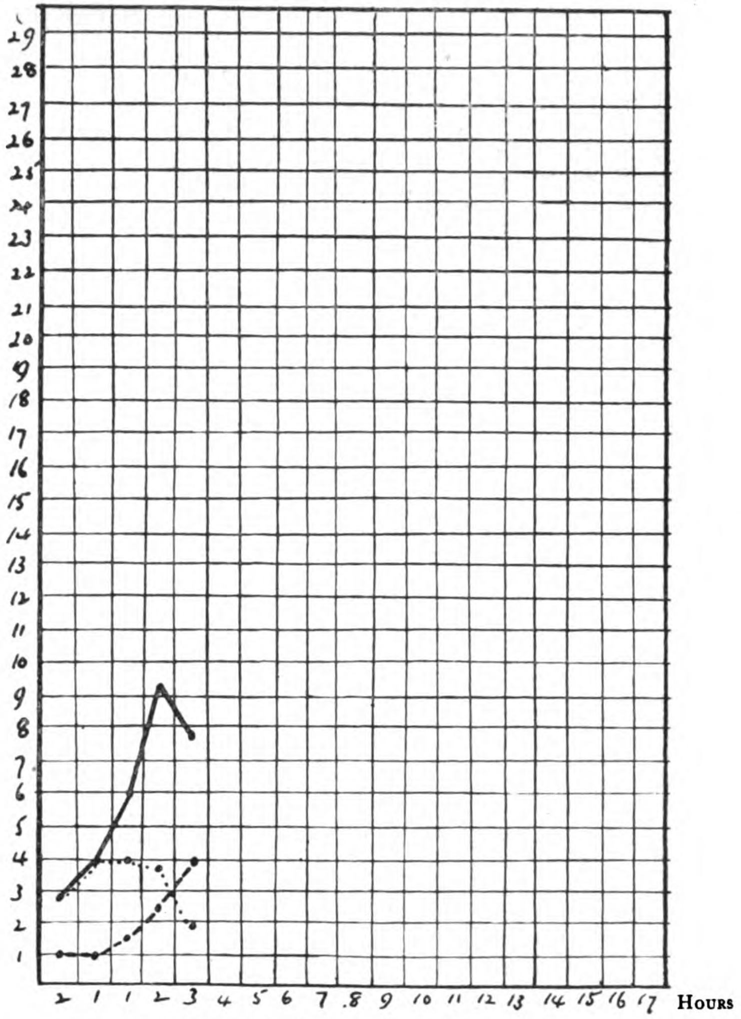


Amount in c.c. of bile passed per hour per kilo. of body weight ....

Relative concentration of bile pigment – – – – –

Relative amount of bile pigment ▬▬▬

Graph V.—Giving the results of hourly examinations of the bile as regards volume and concentration of pigment of Rabbit 15 which received intravenously 13·2 c.c. per kilo. of 50 per cent. haemoglobin solution (6·6 g. Hb.).



Amount in c.c. of bile passed per hour per kilo. of body weight ....

Relative concentration of bile pigment – – – – –

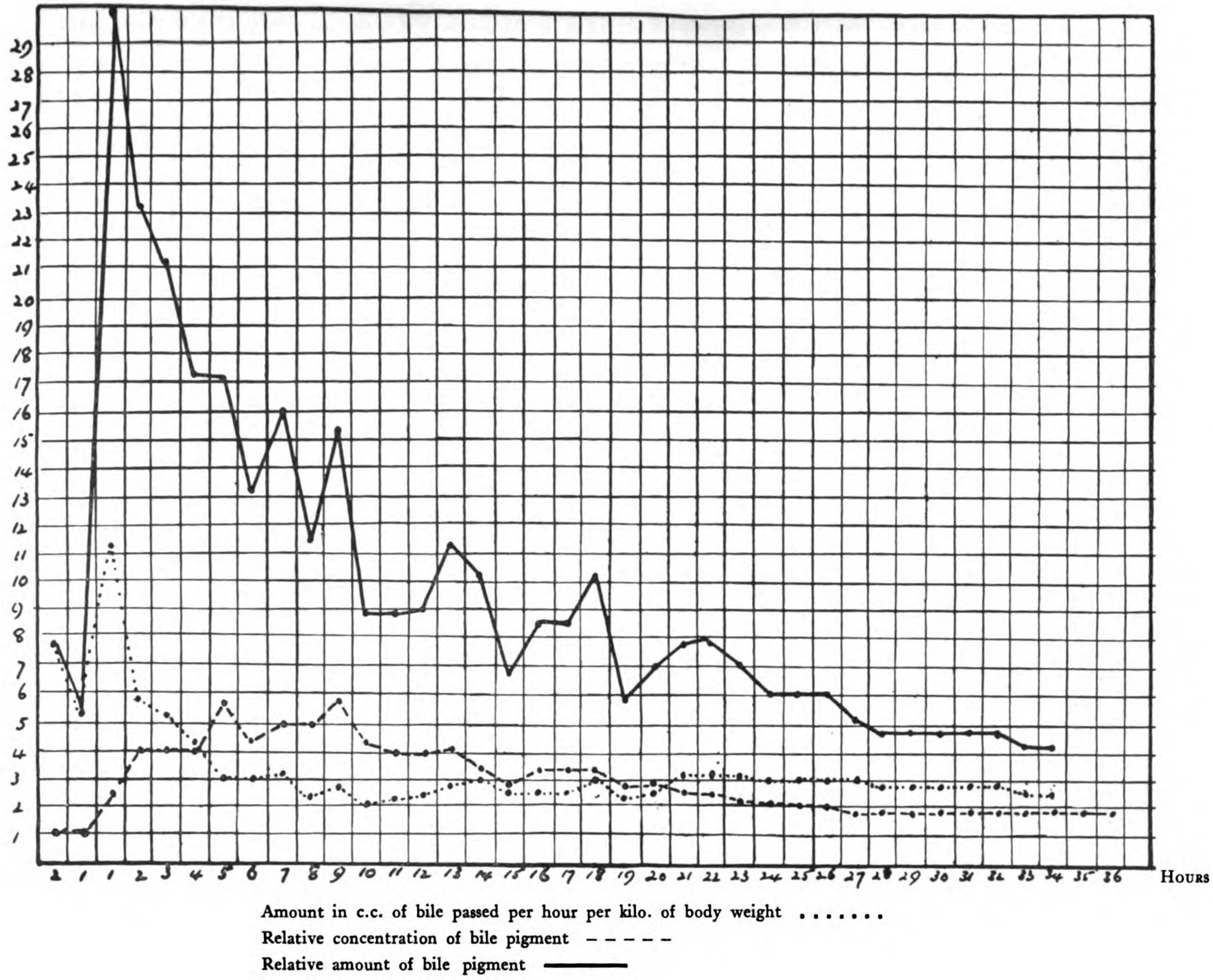
Relative amount of bile pigment ▬▬▬

The third curve on the graphs, indicated by the continuous line, represents the relative amount per kilogramme of body weight of bile pigment passed from hour to hour. It represents the product obtained by multiplying the number of cubic centimetres of bile passed each hour by the relative concentration of pigment present. In the control experiments these curves gradually fall until the 18th to 20th hour after injection of the normal saline solution, when in the case of Rabbit 6 there is a fairly marked rise up to a level representing about two and a half times the amount of pigment noted in the first samples collected. This rise corresponds to the increase in concentration of pigment, but is to a certain extent masked by the diminution in quantity of bile passed. No such increase is seen in Rabbit 5, where the fall in the volume of bile counteracts the increase in concentration of pigment. In those animals which received an intravenous injection of haemoglobin there is an immediate increase in the output of bile pigment varying from four to six times that of the first specimen. The curve representing the relative quantity of pigment excreted reaches its maximum within two or three hours following the introduction of the haemoglobin, and subsequently gradually falls until in from 10 to 14 hours it has reached its initial level. Occasionally, as in the controls, there was a second rise corresponding to the late increase of the concentration of pigment, but frequently this increase in concentration was not sufficient to balance the diminution of the volume of bile passed, so that the curve representing the relative amount of pigment excreted continued to fall gradually until the death of the animal.

The immediate increase in the output of bile pigment following the introduction of haemoglobin solution into the circulation, and its absence after the injection of physiological saline solution in the earlier series of experiments are clearly shown in Charts 7 and 8 respectively, but owing to the fact that the samples were collected, and examinations made, after the lapse of varying intervals of time the graphs are not so regular as in the experiments already described.

Referring to the question of the cause of the increase in concentration of bile pigment, which appears to be a constant phenomenon in all the experiments some 18 to 20 hours after the operation of

GRAPH VI.—Giving the results of hourly examinations of the bile as regards volume and concentration of pigment of Rabbit 16 which received intravenously 14 c.c. per kilo. of 50 per cent. solution of haemoglobin (7 g. Hb.).



ligaturing the bile duct and inserting the cannula into the gall bladder, it appears as if the increase in concentration of pigment were the result of a diminution in the volume of bile passed. Such a condition of affairs is frequently observed in the case of urine; when for any reason the volume excreted diminishes the relative concentration of solid material is usually increased. However, it must be observed that although during the first 18 hours of the experiment the quantity of bile excreted per hour frequently varied considerably—during certain hours it was sometimes twice as great as during other equal periods—nevertheless in the control experiments, in which either there was no injection or merely physiological salt solution, the concentration of pigment remained remarkably constant.

Summarising the data obtained in this investigation, we find that consequent upon intravenous injection of haemoglobin solution there is a distinct and immediate increase not only in the concentration of bile pigment, but also in the amount of bile pigment excreted. During the three or four hours following the injection of haemoglobin the amount of pigment excreted may increase to from four to six times the amount found in the samples examined before the injection. Two hypotheses may be advanced to explain this increase in amount of pigment excreted, first that the haemoglobin injected is actually converted by the liver into bile pigment, or secondly that it merely stimulates the liver cells to an increased production of bile pigment. Against the former hypothesis is the fact that the increase of pigment is not so great as might be anticipated from the relatively large quantities of haemoglobin introduced into the circulation and consequent high degree of haemoglobinaemia resulting (up to 20 %). The amount of haemoglobin solution found in the plasma of normal rabbits is exceedingly small, varying from ·02 to ·05 %. This fact seems to indicate that the degree of red cell destruction normally occurring in the living animal is slight. Assuming that the pigment of the normal bile is derived entirely from haemoglobin set free by the disintegration of erythrocytes normally occurring in the living animal, then it is rather surprising that when the degree of haemoglobinaemia is increased 400 times the amount of bile pigment should only be increased at the most sixfold.

Chart VII.—Giving the relative amounts of bile pigment passed per hour in Rabbits 7 to 12, all of which had received haemoglobin intravenously.

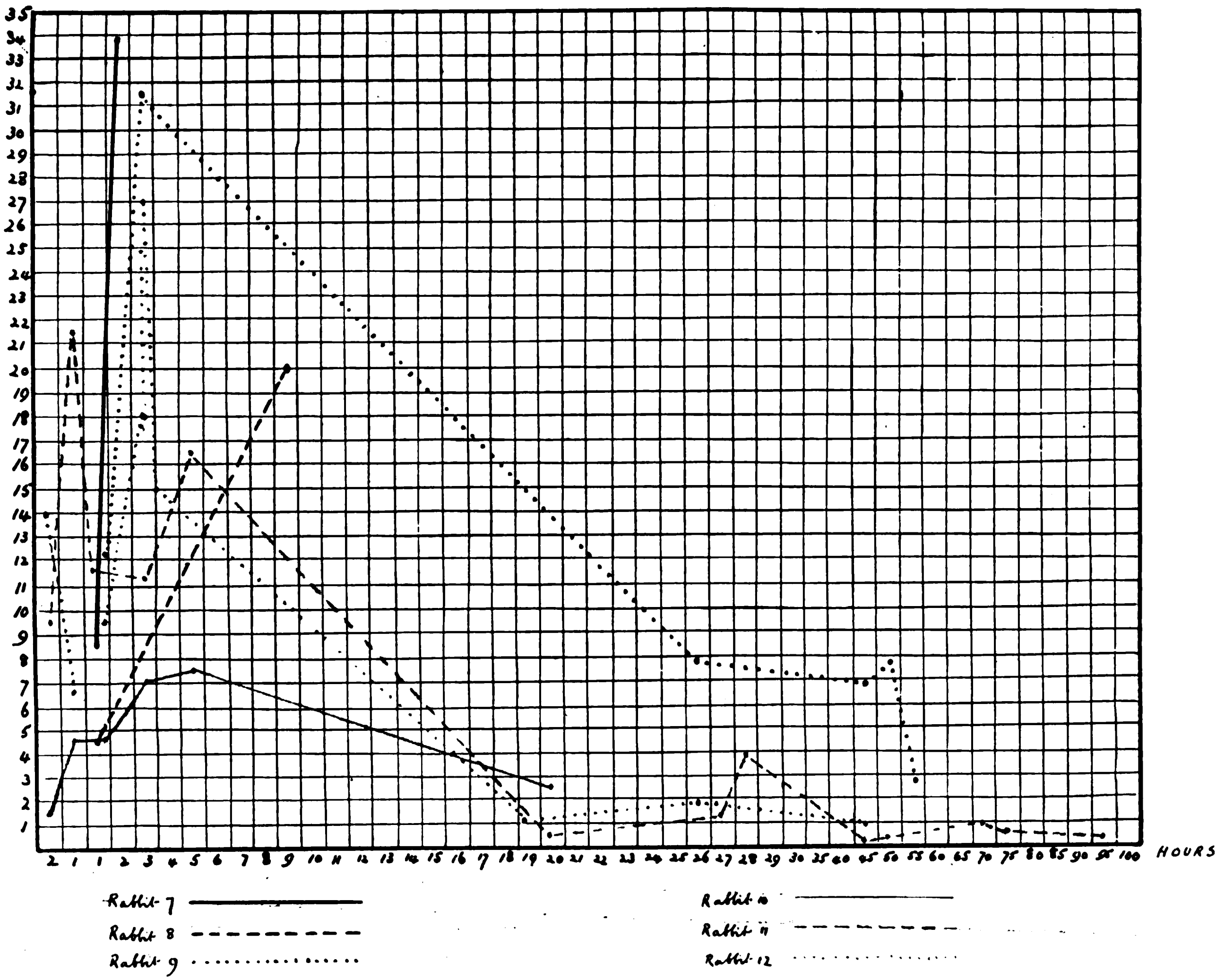
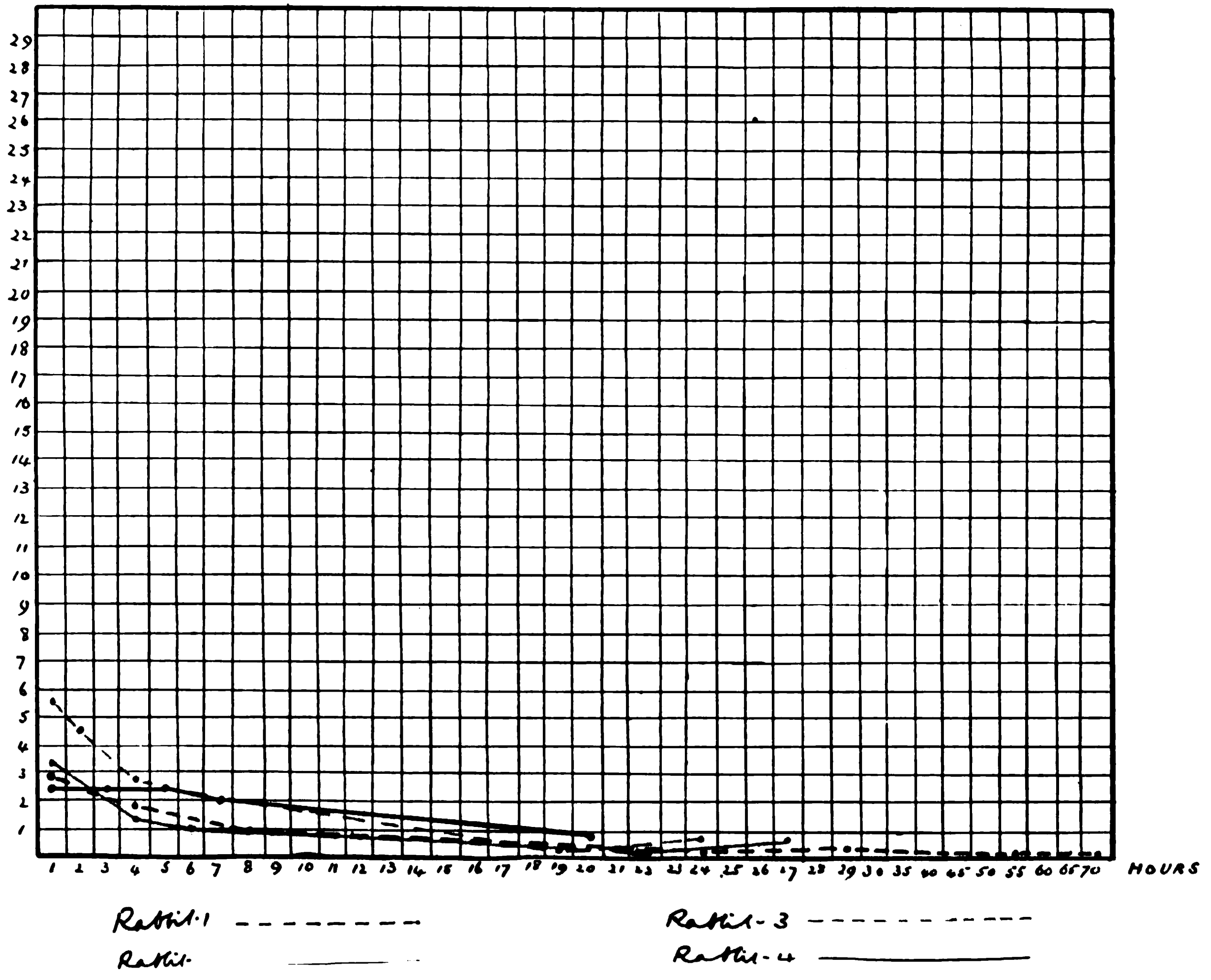


CHART VIII.—Giving the relative amounts of bile pigment passed per hour in Rabbits 1 to 4. None of the animals received any intravenous injection except Rabbit 4 in which 15 c.c. per kilo. of ·9 per cent. NaCl solution was introduced intravenously.



The work of Yorke and Nauss* showed that the mere introduction of homologous haemoglobin into the veins of normal rabbits was not sufficient to produce jaundice. No icterus of the skin or conjunctivae was observed in an animal which had received intravenously 27 gm. of haemoglobin per kilogramme of body weight. The haemoglobinaemia resulting from such injections gradually disappeared, leaving the blood plasma clear and colourless without any trace of bile pigment. Hence, although from these experiments it appears that intravenous injection of haemoglobin results in an increased excretion of bile pigment, the icterus almost invariably to be observed in blackwater fever cannot be attributed solely to haemoglobinaemia resulting from the great disintegration of red blood cells usually associated with this disease.

---

* loc. cit.

TABLE 1.—Giving details of examination of bile in Rabbit 1; no injection was given.

Time after insertion of cannula into gall bladder	Amount of bile passed	Amount of bile passed per kilo. of body weight per hour	Relative concentration of bile pigment	Relative amount of bile pigment passed per kilo. of body weight per hour	Remarks
1¼ hours ...	6·0	2·8	1·0	2·8	
4¾ ,, ...	10·0	1·8	1·0	1·8	
7¾ ,, ...	6·0	1·2	·8	·96	
24 ,, ...	6·5	·23	·7	·15	
29 ,, ...	2·0	·23	1·0	·23	
53 ,, ...	·75	·002	2·0	·004	
71 ,, ...	3·5	·12	5·7	·68	Animal in feeble condition
78 ,, ...	·75	·01	—	—	Killed.

TABLE 2.—Giving details of examination of bile in Rabbit 2; no injection was given.

Time after insertion of cannula into gall bladder	Amount of bile passed	Amount of bile passed per kilo. of body weight per hour	Relative concentration of bile pigment	Relative amount of bile pigment passed per kilo. of body weight	Remarks
1¼ hours ...	8·0	3·2	1·0	3·2	
3¾ ,, ...	6·0	1·2	1·0	1·2	
6½ ,, ...	8·0	1·3	·8	1·0	
22½ ,, ...	7·0	·22	·7	·15	
27½ ,, ...	3·0	·3	1·0	·3	
51 ,, ...	·75	·01	2·4	·02	Animal died.

Table 3.—Giving details of examination of bile in Rabbit 3; no injection was given.

Time after insertion of cannula into gall bladder	Amount of bile passed	Amount of bile passed per kilo. of body weight per hour	Relative concentration of bile pigment	Relative amount of bile pigment passed per kilo. of body weight per hour	Remarks
¾ hour ...	12·0	5·4	1·0	5·4	
2 hours ...	10·3	4·4	1·0	4·4	
3½ ,, ...	7·0	2·6	1·1	2·6	
19½ ,, ...	5·0	·15	1·0	·15	
24 ,, ...	3·0	·33	1·7	·56	
47 ,, ...	1·5	·04	2·5	·1	
52 ,, ...	—	—	—	—	Animal killed.

Table 4.—Giving details of examination of bile in Rabbit 4; sodium chloride solution (·9%) injected.

Time	Amount of bile passed	Amount of bile passed per kilo. of body weight per hour	Relative concentration of bile pigment	Relative amount of bile pigment passed per kilo. of body weight per hour	Remarks
1 hour ...	4·0	2·4	1·0	2·4	NaCl injected (15 c.c. of ·9% solution per kilo. of body weight).
3 hours ...	8·0	2·4	1·0	2·4	
4½ ,, ...	4·0	2·4	1·0	2·4	
5½ ,, ...	4·0	2·4	·83	2·0	
21½ ,, ...	40·0	1·5	1·25	1·7	
27½ ,, ...	3·0	·5	1·6	·8	Animal died.

Table 5.—Giving details of examination of bile in Rabbit 5; sodium chloride solution (·9%) injected.

Time	Amount of bile passed in c.c. per hour	Amount of bile passed in c.c. per hour per kilo. of body weight	Relative concentration of bile pigment	Relative amount of bile pigment per hour per kilo. of body weight	Remarks
4th hr. before inj.	8·5	6·4	—	6·4	Bile contained blood.
3rd ,, ,,	6·0	4·5	1	4·5	
2nd ,, ,,	5·25	4·0	1	4·0	
1st ,, ,,	5·25	4·0	1	4·0	NaCl injected (12 c.c. of ·9% solution per kilo. of body weight.)
1st hr. after inj.	10·0	7·5	1	7·5	
2nd ,, ,,	10·0	7·5	1	7·5	
3rd ,, ,,	6·25	4·7	1	4·7	
4th ,, ,,	6·5	4·7	1	4·7	Ate turnip well.
5th ,, ,,	7·0	5·2	1	5·2	
6th ,, ,,	6·0	4·5	1	4·5	
7th ,, ,,	5·75	4·3	1·08	4·6	
8th ,, ,,	5·5	4·1	1·08	4·4	
9th ,, ,,	5·25	3·9	1·08	4·2	
10th ,, ,,	4·25	3·2	1·08	3·6	
11th ,, ,,	4·6	3·4	1·08	3·7	
12th ,, ,,	4·3	3·2	1·2	3·8	
13th ,, ,,	3·9	2·9	1·25	3·6	
14th ,, ,,	3·4	2·5	1·25	3·1	
15th ,, ,,	4·6	3·4	1·25	4·2	
16th ,, ,,	3·3	2·9	1·25	3·1	Ate a little turnip.
17th ,, ,,	2·6	1·9	2·0	3·8	
18th ,, ,,	3·2	2·4	2·0	4·8	
19th ,, ,,	3·2	2·4	2·0	4·8	
20th ,, ,,	3·0	2·2	1·8	4·0	Ate Oats well.
21st ,, ,,	2·75	2·0	1·8	4·0	
22nd ,, ,,	2·75	2·0	1·8	4·0	
23rd ,, ,,	2·5	1·9	1·8	3·4	
24th ,, ,,	2·25	1·7	1·8	3·0	
25th ,, ,,	2·0	1·5	1·8	2·7	
26th ,, ,,	1·5	1·1	1·8	2·0	
27th ,, ,,	1·5	1·1	1·8	2·0	Refused food.
28th ,, ,,	0·75	0·56	1·8	1·0	
29th ,, ,,	0·55	0·41	1·8	0·74	
30th ,, ,,	0·5	0·37	1·8	0·67	
31st ,, ,,	0·3	0·22	1·8	0·40	
32nd ,, ,,	0·3	0·22	1·8	0·40	
33rd ,, ,,	0·3	0·22	1·8	0·40	
34th ,, ,,	0·25	0·19	1·8	0·34	
35th ,, ,,	0·25	0·19	1·8	0·34	
36th ,, ,,	0·25	0·19	1·8	0·34	
37th ,, ,,	0·15	0·11	1·8	0·20	
38th ,, ,,	0·15	0·11	1·8	0·20	
39th ,, ,,	0·15	0·11	1·8	0·20	
40th ,, ,,	0·125	0·09	1·8	0·17	
41st ,, ,,	0·125	0·09	1·8	0·17	Animal very weak; killed.

Table 6.—Giving details of examination of bile in Rabbit 6 ; sodium chloride solution (·9%) injected.

Time	Amount of bile passed in c.c. per hour	Amount of bile passed in c.c. per hour per kilo. of body weight	Relative concentration of bile pigment	Relative amount of bile pigment per kilo. of body weight	Remarks
2nd hr. before inj.	7·5	5·4	1·0	5·4	
1st ,,	6·25	4·8	1·0	4·8	NaCl injected (12 c.c. of ·9% solution per kilo. of body weight).
1st hr. after inj.	8·25	6·0	1·0	6·0	
2nd ,,	8·25	6·0	1·0	6·0	
3rd ,,	6·0	4·3	1·0	4·3	
4th ,,	6·0	4·3	1·0	4·3	
5th ,,	6·0	4·3	1·0	4·3	
6th ,,	6·0	4·3	1·0	4·3	
7th ,,	5·0	3·6	1·0	3·6	Ate turnip well.
8th ,,	5·25	3·7	1·0	3·7	
9th ,,	4·75	3·4	1·0	3·4	
10th ,,	4·25	3·0	1·0	3·0	
11th ,,	4·6	3·3	1·1	3·6	
12th ,,	4·9	3·5	1·1	3·8	
13th ,,	4·3	3·1	1·1	3·4	
14th ,,	4·4	3·1	1·3	4·0	
15th ,,	4·4	3·1	1·3	4·0	
16th ,,	6·3	4·7	1·3	6·1	
17th ,,	3·5	2·5	2·2	5·5	Ate turnip.
18th ,,	2·4	1·7	2·4	4·1	
19th ,,	2·9	2·1	2·9	6·1	
20th ,,	3·3	2·2	3·3	7·3	
21st ,,	3·3	2·2	3·3	7·3	
22nd ,,	4·0	2·8	3·3	9·2	
23rd ,,	3·75	2·7	3·3	8·9	Ate a little oats.
24th ,,	3·5	2·5	3·7	8·2	
25th ,,	3·75	2·7	3·9	8·9	
26th ,,	2·75	2·0	3·9	7·8	
27th ,,	2·75	2·0	3·9	7·8	
28th ,,	2·75	2·0	3·9	7·8	
29th ,,	3·25	2·2	3·9	7·2	Ate turnip well.
30th ,,	3·25	2·2	3·9	7·2	
31st ,,	3·25	2·2	3·9	7·2	
32nd ,,	1·5	1·16	3·9	4·5	
33rd ,,	5·25	4·0	3·9	15·6	
34th ,,	2·0	1·6	3·9	6·2	
35th ,,	2·0	1·6	3·9	6·2	
36th ,,	3·0	2·4	3·9	9·3	
37th ,,	2·0	1·6	3·9	6·2	
38th ,,	2·0	1·6	3·9	6·2	
39th ,,	4·0	3·4	3·9	13·3	
40th ,,	2·0	1·6	3·9	6·2	Ate turnip well.
41st ,,	2·6	2·1	3·9	8·2	
42nd ,,	2·6	2·1	3·9	8·2	
43rd ,,	3·0	2·4	3·9	9·3	
44th ,,	3·0	2·4	3·9	9·3	
45th ,,	2·5	2·0	3·9	7·8	
46th ,,	2·5	2·0	3·9	7·8	
47th ,,	2·5	2·0	3·9	7·8	
48th– 53rd ,,	2·8	2·4	4·0 (approx.)	9·6 (approx.)	
54th– 59th ,,	2·1	1·7	4·1 (approx.)	7·0 (approx.)	Animal eating well.
60th– 65th ,,	1·4	1·1	4·2 (approx.)	4·4 (approx.)	
66th– 71st ,,	2·6	2·1	Could not be determined	Could not be determined	Bile brownish green colour, absorption band present in position of methaemoglobin band.
72nd– 77th ,,	2·2	1·8	,,	,,	
78th– 83rd ,,	2·25	1·8	,,	,,	
84th– 89th ,,	1·0	0·85	,,	,,	
90th– 95th ,,	2·0	1·67	,,	,,	
96th–101st ,,	1·9	1·58	,,	,,	
102nd–107th ,,	2·0	1·67	,,	,,	Condition fairly good.
113th ,,	—	—	—	—	Animal killed.

TABLE 7.—Giving details of examination of bile in Rabbit 7 ; haemoglobin solution injected.

Time	Amount of bile passed	Amount of bile passed per kilo. of body weight per hour	Relative concentration of bile pigment	Relative amount of bile pigment passed per kilo. of body weight per hour	Remarks
¼ hr. before inj.	2·5	—	—	—	Bile light yellowish green colour.
¼ hr. after inj.	3·8	8·8	1·0	8·8	Hb. injected (20 c.c. of 50% solution = 8·5 g. hb. per kilo. of body weight.)
½ ,, ,,	4·0	—	—	—	
¾ ,, ,,	3·8	—	—	—	
1 ,, ,,	4·0	9·2	—	—	Bile dark green in colour.
1½ ,, ,,	7·0	—	—	—	
2 ,, ,,	5·0	6·25	5·4	33·75	Convulsions and death 5 mins. after a second injection of Hb. (2 g.)
2½ ,, ,,	—	—	—	—	

TABLE 8.—Giving details of examination of bile in Rabbit 8 ; haemoglobin solution injected.

Time	Amount of bile passed	Amount of bile passed per kilo. of body weight per hour	Relative concentration of bile pigment	Relative amount of bile pigment passed per kilo. of body weight per hour	Remarks
¾ hr. after inj.	3·0	4·4	1·0	4·4	Hb. injected (24 c.c. of 50% solution = 13·3 g. hb. per kilo. f body weight.)
3¼ hrs. ,,	2·0	2·2	—	—	
4¼ ,, ,,	3·0	3·3	—	—	
5¼ ,, ,,	3·0	3·3	—	—	
6¼ ,, ,,	3·0	3·3	—	—	
9¼ ,, ,,	5·0	2·0	10·0	20	
9½ ,, ,,	—	—	—	—	Convulsions and death 5 mins. after a second injection of hb. (3 g.)

Table 9.—Giving details of examination of bile in Rabbit 9; haemoglobin solution injected.

Time	Amount of bile passed	Amount of bile passed per kilo. of body weight per hour	Relative concentration of bile pigment	Relative amount of bile pigment passed per kilo. of body weight per hour	Remarks
½-¼ hr. before inj.	6·0	12·2	1·0	12·2	Bile light yellowish green colour.
¼ hr. before to ¼ hr. after inj.	3·25	—	—	—	Hb. injected (40 c.c. of 50% solution = 17·5 g. hb. per kilo. of body weight.)
½ hr. after inj.	12·0	—	—	—	
¾ hrs. ,,	6·0	22·8	—	—	
1 hr. ,,	8·0	—	—	—	
1¼ hrs. ,,	6·0	—	—	—	
1¾ ,, ,,	6·0	17·5	—	—	
2 ,, ,,	10·0	—	—	—	
2½ ,, ,,	6·5	—	—	—	
2¾ ,, ,,	5·5	10·5	3·0	31·5	
4¾ ,, ,,	5·0	2·4	—	—	
5¾ ,, ,,	6·0	5·2	—	—	
20 ,, ,,	12·0	·75	—	—	
22 ,, ,,	2·5	1·1	—	—	Bile very dark green in colour.
26 ,, ,,	3·0	·65	12·0	7·8	
43 ,, ,,	11·0	·56	12·0	6·7	Animal ate a little cabbage.
48 ,, ,,	3·5	·65	12·0	7·8	
57 ,, ,,	3·5	·34	7·8	2·6	Animal found dead Post-mortem: lungs healthy, left lobe of liver covered with thin layer of lymph, peritoneum elsewhere free from lymph or fluid, urinary bladder empty, kidneys large and chocolate in colour, cut surface shows numerous brown striae.

TABLE 10.—Giving details of examination of bile in Rabbit 10 ; haemoglobin solution injected.

Time	Amount of bile passed	Amount of bile passed per kilo. of body weight per hour	Relative concentration of bile pigment	Relative amount of bile pigment passed per kilo. of body weight per hour	Remarks
4-1¼ hrs. before inj.	5·0	1·2	1·0	1·2	Bile yellowish green colour.
1¼-¾ hrs. before inj.	7·0	4·5	1·0	4·5	
¾ hr. before to ¼ hr. after inj.	3·0	4·5	1·0	4·5	Hb. injected (25 c.c. of 50% solution = 8 g. Hb. per kilo. of body weight).
1½ hrs. after inj.	7·0	3·0	1·5	4·5	
3 ,, ,,	8·0	3·5	2·0	7·0	
5 ,, ,,	9·5	3·0	2·5	7·5	Bile very dark green.
20 ,, ,,	19·0	0·8	3·0	2·4	
24 ,, ,,	0·5	0·07	—	—	Animal died.

TABLE 11.—Giving details of examination of bile in Rabbit 11 ; haemoglobin solution injected.

Time	Amount of bile passed	Amount of bile passed per kilo. of body weight per hour	Relative concentration of bile pigment	Relative amount of bile pigment passed per kilo. of body weight per hour	Remarks
2-1¼ hrs. before inj.	7·5	9·5	1·0	9·5	Bile contained a little blood.
1¼-¾ hrs. before inj.	14·0				Bile greenish yellow in colour.
¾ hr. before to time of inj.	18·0	21·3	1·0	21·3	Hb. injected (30 c.c. of 50% solution = 8·3 g. hb. per kilo. of body weight).
Time of inj. to ¼ hr. after	14·0				
1¼ hrs. after inj.	12·0	6·7	1·75	11·7	
3¼ ,, ,,	13·5	3·75	3·0	11·2	Bile dark green in colour.
4¾ ,, ,,	9·0	3·3	5·0	16·5	,, ,,
20 ,, ,,	4·0	0·15	4·5	0·67	
27 ,, ,,	3·0	0·25	5·0	1·25	,, ,,
28 ,, ,,	2·5	1·6	2·4	3·8	
44 ,, ,,	1·5	0·05	1·5	0·075	
51 ,, ,,	1·0	0·08	1·2	0·09	5 c.c. of ruby red urine passed .
68 ,, ,,	16·0	0·5	1·5	0·75	
74 ,, ,,	5·5	0·5	1·0	0·5	
92 ,, ,,	8·0	0·25	1·0	0·25	Animal very weak, refused food.
140 ,, ,,	—	—	—	—	Animal killed.

Table 12.—Giving details of examination of bile in Rabbit 12 ; haemoglobin solution injected.

Time	Amount of bile passed	Amount of bile passed per kilo. of body weight per hour	Relative concentration of bile pigment	Relative amount of bile pigment passed per kilo. of body weight per hour	Remarks
2¼–1¾ hrs. before inj.	12·0	14·0	1·0	14·0	Bile light yellowish green colour.
1¾ hrs. to time of inj.	9·7	3·25	2·0	6·5	Hb. injected (30 c.c. of 50% solution = 9 g. hb. per kilo. of body weight).
Time of inj. to 1 hr. after	9·5	6·5	1·5	9·75	
1¾ hrs. after inj. ...	11·5	10·8	2·5	27·0	
3 ,, ,,	11·0	6·0	2·5	15·0	Animal ate a little cabbage.
19 ,, ,,	5·0	0·2	6·25	1·25	Bile very dark green in colour.
26 ,, ,,	1·5	0·15	12·5	1·87	
33 ,, ,,	1·5	0·05	16·2	0·8	Animal died.

Table 13.—Giving details of examination of bile in Rabbit 13 ; haemoglobin solution injected.

Time	Amount of bile passed in c.c. per hour	Amount of bile passed in c.c. per hour per kilo. of body weight	Relative concentration of bile pigment	Relative amount of bile pigment per hour per kilo. of body weight	Remarks
3rd hr. before inj.	7·0	4·7	1·0	4·7	
2nd ,,	9·0	6·0	1·0	6·0	
1st ,,	9·25	6·2	1·0	6·2	Hb. injected (5 c.c. of 50% solution = 1·6 g. hb. per kilo of body weight.)
1st hr. after inj.	7·0	4·7	1·0	4·7	
2nd ,,	4·75	3·2	1·25	4·0	
3rd ,,	4·5	3·0	2·66	7·0	
4th ,,	4·5	3·0	4·0	12·0	
5th ,,	4·75	3·2	3·1	9·9	
6th ,,	3·75	2·5	3·1	7·75	
7th ,,	3·5	2·3	3·1	7·13	
8th ,,	3·0	2·0	3·1	6·2	
9th ,,	3·0	2·0	3·1	6·2	
10th ,,	3·0	2·0	3·1	6·2	
11th ,,	2·75	1·8	3·1	5·58	
12th ,,	3·0	2·0	2·0	4·0	
13th ,,	3·0	2·0	2·0	4·0	
14th ,,	2·5	1·7	2·0	3·4	
15th ,,	2·0	1·3	2·0	2·6	
16th ,,	2·0	1·3	2·0	2·6	
17th ,,	2·0	1·3	2·0	2·6	
18th ,,	2·0	1·3	3·0	3·9	
19th ,,	·75	·5	3·0	1·5	
20th ,,	1·5	1·0	3·0	3·0	
21st ,,	1·75	1·2	3·0	3·6	
22nd ,,	1·0	·7	3·0	2·1	
23rd ,,	·75	·5	3·0	1·5	
24th ,,	·75	·5	3·0	1·5	
25th ,,	·75	·5	3·0	1·5	
26th ,,	·75	·5	3·0	1·5	
27th ,,	·75	·5	3·0	1·5	
28th-33rd ,,	·5	·3	3·75	1·125	
34th-39th ,,	·5	·3	3·75	1·125	Animal, which had refused food and looked ill, was killed.

TABLE 14.—Giving details of examination of bile in Rabbit 14 ; haemoglobin solution injected.

Time	Amount of bile passed in c.c. per hour	Amount of bile passed in c.c. per hour per kilo. of body weight	Relative concentration of bile pigment	Relative amount of bile pigment per hour per kilo. of body weight	Remarks
3rd hr. before inj.	4·0	2·25	·8	1·8	
2nd ,, ,,	4·0	2·25	1·3	2·9	
1st ,, ,,	6·0	3·4	1·0	3·4	Hb. injected (18 c.c. of 50% solution = 4·1 g. hb. per kilo. of body weight.)
1st hr. after inj.	4·0	2·25	1·5	3·4	
2nd ,, ,,	6·0	3·4	3·1	10·5	
3rd ,, ,,	3·5	2·0	3·1	6·2	Secretion of bile ceased half hour before death which was accompanied by violent convulsions.

TABLE 15.—Giving details of examination of bile in Rabbit 15 ; haemoglobin solution injected.

Time	Amount of bile passed in c.c. per hour	Amount of bile passed in c.c. per hour per kilo. of body weight	Relative concentration of bile pigment	Relative amount of bile pigment per hour per kilo. of body weight	Remarks
2nd hr. before inj.	4·25	2·8	1·0	2·8	
1st ,, ,,	6·0	4·0	1·0	4·0	Hb. injected (20 c.c. of 50% solution = 6·6 g. hb. per kilo. of body weight.)
1st hr. after inj.	6·0	4·0	1·5	6·0	
2nd ,, ,,	5·5	3·7	2·5	9·3	
3rd ,, ,,	2·8	1·9	4·0	7·6	Secretion of bile ceased half hour before death which was preceded by violent convulsions.

Table 16.—Giving details of examination of bile in Rabbit 16; haemoglobin solution injected.

Time		Amount of bile passed in c.c. per hour	Amount of bile passed in c.c. per hour per kilo. of body weight	Relative concentration of bile pigment	Relative amount of bile pigment per hour per kilo. of body weight	Remarks
2nd hr. before inj.		5·5	3·7	—	—	Bile contained blood.
1st	,,	9·0	6·0	1·0	6·0	Hb. injected (21 c.c. of 50 % solution = 7 g. hb. per kilo. of body weight).
1st hr. after inj.		8·0	5·3	1·0	5·3	
2nd	,,	8·0	5·5	1·8	9·9	
3rd	,,	8·5	5·8	3·3	19·1	
4th	,,	8·5	5·8	4·0	23·2	
5th	,,	8·0	5·3	4·0	21·2	
6th	,,	6·5	4·3	4·0	17·2	Ate turnip.
7th	,,	4·5	3·0	5·7	17·1	
8th	,,	4·5	3·0	4·4	13·2	
9th	,,	4·75	3·2	5·0	16·0	
10th	,,	3·5	2·3	5·0	11·5	Ate turnip.
11th	,,	4·0	2·7	5·7	15·4	
12th	,,	3·0	2·0	4·4	8·8	
13th	,,	3·25	2·2	4·0	8·8	
14th	,,	3·75	2·5	4·0	9·0	
15th	,,	4·0	2·7	4·2	11·3	
16th	,,	4·5	3·0	3·4	10·2	
17th	,,	3·75	2·5	2·7	6·75	
18th	,,	3·75	2·5	3·4	8·5	
19th	,,	3·75	2·5	3·4	8·5	
20th	,,	4·5	3·0	3·4	10·2	
21st	,,	3·25	2·2	2·7	5·9	
22nd	,,	3·75	2·5	2·8	7·0	
23rd	,,	4·75	3·2	2·4	7·7	
24th	,,	4·75	3·2	2·4	7·7	Ate bran and oats.
25th	,,	4·75	3·2	2·2	7·0	
26th	,,	4·5	3·0	2·0	6·0	
27th	,,	4·5	3·0	2·0	6·0	
28th	,,	4·5	3·0	2·0	6·0	
29th	,,	4·5	3·0	1·7	5·1	
30th	,,	4·25	2·8	1·7	4·7	
31st	,,	4·25	2·8	1·7	4·7	
32nd	,,	4·25	2·8	1·7	4·7	
33rd	,,	4·25	2·8	1·7	4·7	
34th	,,	4·0	2·7	1·7	4·6	Ate turnip.
35th	,,	3·75	2·5	1·7	4·25	
36th	,,	3·75	2·5	1·7	4·25	
37th–43rd	,,	3·25	2·3	1·7	3·2	
44th–49th	,,	3·25	2·3	1·7	3·2	
50th–55th	,,	3·95	2·5	—	—	
56th–61st	,,	3·25	2·2	—	—	
62nd–67th	,,	2·5	1·7	—	—	
68th–73rd	,,	2·5	1·7	—	—	
74th–79th	,,	2·25	1·5	—	—	
80th–85th	,,	2·25	1·5	—	—	
86th–91st	,,	2·25	1·5	—	—	
92nd–97th	,,	1·75	1·2	—	—	
98th–103rd	,,	1·75	1·2	—	—	

TABLE 16—Continued.

Time		Amount of bile passed in c.c. per hour	Amount of bile passed in c.c. per hour per kilo. of body weight	Relative concentration of bile pigment	Relative amount of bile pigment per hour per kilo. of body weight	Remarks
104th–109th	hr. after inj.	2·0	1·3	—	—	
110th–115th	,,	2·0	1·3	—	—	
116th–121st	,,	2·0	1·3	—	—	Animal in good condition; eats well.
122nd–127th	,,	1·75	1·2	—	—	
128th–133rd	,,	1·5	1·0	—	—	
134th–139th	,,	1·5	1·0	—	—	
140th–145th	,,	1·5	1·0	—	—	
146th–151st	,,	1·7	1·1	—	—	
152nd–157th	,,	1·8	1·2	—	—	
158th–169th	,,	1·5	1·0	—	—	
170th–181st	,,	1·5	1·0	—	—	
182nd–193rd	,,	1·5	1·0	—	—	
194th–205th	,,	1·7	1·1	—	—	
206th–229th	,,	1·4	·9	—	—	
230th–253rd	,,	1·2	·8	—	—	
254th–277th	,,	1·9	1·3	—	—	
278th–301st	,,	2·0	1·3	—	—	
302nd–325th	,,	2·0	1·3	—	—	Cannula came out of gall bladder; no bile escaped through wound.
326th–349th	,,	—	—	—	—	
350th–373rd	,,	—	—	—	—	
374th–397th	,,	—	—	—	—	
398th–421st	,,	—	—	—	—	Animal died. Post mortem examination, much bile in peritoneal cavity, liver necrosed in places, bile ducts distended.

THE ANTI-MALARIAL CAMPAIGN IN FORMOSA

BY

DR. J. HATORI,

MEDICAL INSPECTOR, GOVERNMENT OF FORMOSA

(*Received for publication* 15 *May*, 1914)

WITH MAP

The campaign against malaria in Formosa was undertaken in 1911, and has been conducted on an extensive scale, under the direct control of Prof. Dr. T. Takaki, Director of the Government Medical School and of the Government Science Institute. Medical Inspectors, Dr. T. Kojima, Dr. S. Mori, and myself have been energetically engaged in the operation, with encouraging results. I read a short paper giving a partial result of the work on the occasion of the Second Congress of the Far Eastern Association of Tropical Medicine, held at Hongkong, January, 1912. Presuming it may prove of some interest to place an account of the anti-malarial work, as carried out in Formosa, before Western readers, I have pleasure in sending the following to your journal.

I. OUTLINE OF THE ANTI-MALARIAL MEASURES ADOPTED IN FORMOSA

The anti-malarial measures adopted by the Government have for their principal object the extermination of malarial plasmodia carried by men, by means of medical treatment. Any malaria-stricken locality may be directed to adopt the measure by order, and then the operations are performed there in the following manner. All the inhabitants and sojourners of the locality are summoned up en masse on the day appointed. By means of a puncture of the lobe of the ear, a thin blood film preparation is obtained from everybody. After having been stained with diluted Giemsa's stain, the blood films are subjected to microscopical examination, with a view to finding malarial parasites. To the parasite-carriers thus discovered is administered compulsorily a quinine preparation for a period of thirty days during two months, after Nocht's, or a modified method. The quinine preparations employed are muriate of quinine in tablet (0·2 gr.), euchinine powder (for the use of children and in some other necessary cases), and 'Esanopheles' pills (for cases of splenomegaly). These are given gratis to the plasmodia-carriers in the presence of the police officials. When anti-malarial work is to be commenced in a locality, we, as executive officers, proceed to the place, accompanied by assistants, and fully equipped with medicine, necessaries, etc. There, we set up an anti-malarial station, and then we examine the microscopical preparations with great care, each expert being able to examine about eighty films per diem. All the housekeepers in such localities are given orders to use mosquito nets by night, and to keep clean their premises. On the other hand, the 'Bōekikumiai'* and the 'Ho-kō,' or the self-governing corporations, have the obligation of keeping clean the area. And the duty of controlling the inhabitants and of treating the parasite-carriers falls on the police officials.

It is, then, a matter of course that our anti-malarial operation can be brought to a successful issue through the conjoint endeavours of the active self-governing corporations and the competent local authorities. Some Local Governments, such as Taihoku-Chō and Taichu-Chō, which have sufficient means at their disposal have undertaken operations on the lines above stated. Previous to the beginning of the work (April, 1911) the Civil Government issued to the Local Authorities a special notification in regard to malaria prevention.

To propagate some knowledge to the people of the nature and prevention of malaria, the Government distributed printed matter written in Japanese and Chinese. And we gave, now and then, lectures and lantern demonstrations in the principal malarious localities.

---

*The 'Bōeki-kumiai' is a corporation composed of Japanese (and Formosans) in a town or city, with the express object of checking epidemic diseases. It was first organized in 1908 in accordance with the Government Order issued, and has proved a successful agent in eradicating plague. Malaria being declared an endemic disease, to be treated as plague, in 1911, the inhabitants of the designated localities were ordered by the Government to organize the said corporation for malaria-prevention. The corporations are allowed to collect the funds needed for cleaning and so on. Householders, families and employees who neglect the preventive regulations, such as the administration of drugs, examination of blood, the use of mosquito-net, the cleaning of premises, etc., shall be fined by the corporation concerned.

The 'Ho-kō' is a self-governing system that has long existed among Formosans. A 'Kō' consists of 10 native households and a 'Ho' of 10 Kō, i.e., 100 households. In former times, this system used to be a very important organization in protecting the inhabitants from robbery and many other risks and dangers. It is still regarded as a helpful organ in the local administration.

II LOCALITIES WHERE THE ANTI-MALARIAL MEASURE WAS ADOPTED, AND THE NUMBER OF PERSONS EXAMINED, ETC.

The localities* where the measure was adopted, and their inhabitants, are as follow: —

TABLE I.

Part	Local Government	Locality	Average population in		Population in 1913
			1911	1912	
N.	Taihoku	1. Hokutō	—	—	1,758 April
		2. Kinpōri	976	1,072	1,164 „
		3. Shōkirun	547	554	564 „
		4. Ginaryō	967	961	974 „
W.	Taichu	5. Inrin	3,486	3,553	3,274 July
		6. Tōseikaku	—	—	3,733 „
	Kagi	7. Chikutōki	1,870	1,652	1,810 Apri
		8. Shōnoryō	159	—	—
		9. Rokumasan	—	171	—
		10. Ensuikō	—	6,572	6,574 April
S.	Akō	11. Akō	5,400	5,997	6,396 April
		12. Banshiho	—	984	1,202 „
	Tainan	13. Hōsan	6,882	6,758	6,729 April
		14. Tapani	—	—	808 „
E.	Karenkō	15. Karenkō	2,836	3,537	3,917 April
		16. Rigyobi	—	88	134 „
		17. Yoshino	—	1,160	—
		18. Toyoda	—	108	—
		19. Kada	—	170	—
		20. Bokusekikaku	1,001	1,115	1,285 April
		21. Shinnun	—	127	—
	Taitō	22. Kashōtō .... (Samasana I.)	—	—	325 May
			Total	....	40,647

Japanese 7,810

Formosan 32,837

---

* See map of Formosa at the end of this paper.

I was engaged in the work on the Eastern coast (and Northern part), Dr. S. Mori worked for about one year in the Western part, Dr. Kojima attended to the work until April last in the Southern part of the Island.

In these localities everybody had to have his blood examined once a month as a rule. In some localities, where the carriers are mostly children, young men under 20 years of age have been monthly examined, while men above 20 years of age have been examined quarterly. In such a way, Dr. Kojima carried on his work with ease at Akŏ, Hōsan, and Ensuikō, calling the examination as minor and major according to their scales. Such a method is feasible in old native towns, where the majority of adult people have become partly immune from endemic malaria, and only children and new-comers are infected with the disease. In those localities where malaria prevails and the inhabitants are equally attacked, irrespective of race and age, a general examination is required. In the course of successive operations, the considerable decrease of plasmodia-carriers will necessitate only a bi-monthly or quarterly examination of the entire or partial population of such localities.

Since I commenced the preliminary operation at Hokutŏ, in July, 1910, and up to April, 1913, the total number of examinations made in the different localities amounted to 409,355, as the following Table shows. Of this number, 112,007 were among Japanese, the remaining 297,348 were among Formosans, including some Aborigines and Chinese subjects. Of these examinations 11,580 were positive as regards parasites, showing a percentage of 2·83.

The positive cases, together with fever cases and splenomegaly cases, which were treated gratis, amounted to over 20,000, and 14,454 cases of them (3/5 of this number being Formosans) were quininized at the expense of the State Treasury, and the remainder by other offices (Local Government, Railway Department, Industrial Bureau, Sugar Factories). The exact number of the latter is not obtainable, and hence the expenses incurred unknown.

Table II.—Number of Blood Examinations made and the positive findings in the different parts of Formosa.

Part	Local Government	Localities	Number examined			Number of positive blood findings	Rate ‰
			From beginning to March, 1912	From April, 1912, to April, 1913	Total		
N.	Taihoku	1. Hokutō	16,163	17,862	34,025	396	11·64
		2. Kimpōri	8,431	11,428	19,859	269	13·55
		3. Shōkirun	3,851	4,443	8,294	239	28·82
		4. Ginaryō	5,405	9,719	15,124	526	34·78
W.	Taichu	5. Inrin	9,013	11,896	20,909	820	39·22
		6. Tōseikaku	—	—	—	—	—
	Kagi	7. Chikutōki	15,477	15,968	31,445	1.014	32·25
		8. Shonōryō	1,475	—	1,475	40	27·12
		9. Rokumasan ...	—	975	975	27	27·69
		10. Ensuikō	—	21,491	21,491	964	44·86
S	Akō	11. Akō	29,516	33,398	62,914	1,480	23·52
		12. Banshiho	—	7,022	7,022	174	24·78
	Tainan	13. Hōsan	23,553	38,060	61,613	1,884	30·58
		14. Tapani	—	728	728	64	87·91
E.	Karenkō	15. Karenkō	30,562	41,840	72,402	2.248	31·05
		16. Rigyobi	—	1,093	1,093	127	116·19
		17. Yoshino	6,531	13,750	20,281	518	25·54
		18. Toyoda	—	1,456	1,456	193	132·55
		19. Kada	—	2,128	2,128	133	62·5
		20. Bokusekikaku	10,873	14,017	24,890	442	17·76
		21. Shinnun	—	1,231	1,231	22	17·87
	Taitō	22. Kashōtō ...... (Samasana I.)	—	—	?	—	—
		Total	160,850	248,505	409,355	11,580	28·29
		Japanese	42,377	69,630	112,007	2890	25·80
		Formosans ...	118,473	178,875	297,348	8690	29·22

III. RESULT OF ANTI-MALARIAL OPERATIONS

A.—Decrease of Parasite- and Spleen-indices

According to the result of the monthly examinations of inhabitants in the localities where the measure was adopted, a marked decrease of parasite indices was witnessed, as the following Table shows:—

Table III.—Parasite Indices (%) of the localities where the work was carried on.

		Hokuto. I		Kinpori	Shokirun	Ginaryo	Inrin	Toseikaku	Chikutoki	Shonoryo	Rokumasan	Ensuiko	Ako	Banshiho	Hosan	Tapani	Karenko	Rigyobi	Yoshino	Toyoda	Kada	Bokusekikaku	Shinnun	Kashoto
		(a)†	(b)†	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1910	VII	6·8	8·26																					
	VIII	2·56	1·37																					
	IX	1·56	0·17																					
	X	0·56	0·76																					
	XI	0·81	0·68																					
	XII	0·75	—																					
1911	I	0·53	—																					
	II	0·59	—																					
	III	0	0·35																					
	IV	2·29	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3·2	—	—	—	—	3·85	—	—
	V	0·63	—	—	—	—	—	—	7·54	5·88	—	—	6·84	—	—	—	2·0	—	—	—	—	2·20	—	—
	VI	1·14	—	3·95	2·88	—	—	—	10·80	3·07	—	—	•9·15	—	—	—	2·63	—	—	—	—	2·99	—	—
	VII	0·78	0·53	—	—	10·20	—	—	4·56	3·50	—	—	•3·17	—	—	—	3·9	—	—	—	—	5·54	—	—
	VIII	0·53	—	5·54	10·22	—	—	—	6·11	1·33	—	—	3·41	—	9·66	—	3·29	—	—	—	—	3·36	—	—
	IX	1·48	—	2·24	5·52	—	9·66	—	6·31	3·28	—	—	•3·33	—	4·35	—	3·25	—	—	—	—	3·70	—	—
	X	1·74	—	2·51	5·51	11·35	—	—	2·56	2·13	—	—	•3·51	—	4·95	—	4·39	—	—	—	—	3·05	—	—
	XI	4·06	2·30	1·20	6·77	3·33	6·84	—	2·73	2·31	—	—	1·51	—	5·24	—	5·09	—	14·71	—	—	1·63	—	—
	XII	2·06	—	0·97	2·09	2·02	3·93	—	2·71	3·00	—	—	•2·06	—	2·85	—	5·0	—	7·36	—	—	1·57	—	—
1912	I	3·10	—	0·84	2·09	1·77	3·07	—	2·23	3·25	—	—	•2·33	—	2·91	—	5·67	—	1·80	—	—	1·13	—	—
	II	1·56	1·5	0·52	0·47	0·68	1·13	—	1·84	0	—	—	0·99	—	2·47	—	4·43	18·0	2·07	18·25	10·7	1·27	—	—
	III	1·72	—	0·95	1·06	—	1·60	—	1·33	1·00	—	—	•1·87	—	1·65	—	2·65	22·0	2·57	5·4	12·7	0·94	—	—
	IV	0·92	—	0·66	1·27	2·19	0·68	—	0·7	—	—	—	•1·28	—	2·17	—	2·11	7·3	2·08	6·25	5·73	0·6	—	—
	V	0·97	1·33	0·21	0·86	1·13	—	—	1·11	—	—	—	1·20	—	3·21	—	2·11	9·77	0·95	12·37	3·55	0·81	—	—
	VI	1·81	0·79	—	—	—	—	—	—	—	—	—	•2·09	—	2·84	—	2·67	8·1	1·01	7·84	3·92	1·39	6·0	—
	VII	—		1·88	0·77	1·14	—	—	2·78	—	—	—	•2·50	5·80	2·81	—	4·04	11·84	0·96	7·93	9·4	1·38	1·53	—
	VIII	1·17		—	—	6·06	—	—	4·29	—	—	—	1·20	•5·95	2·77	—	2·06	8·2	0·58	12·24	1·4	2·22	1·62	—
	IX	1·43		4·15	3·38	2·82	—	—	2·13	—	—	—	•1·42	•4·60	1·76	—	3·05	13·8	1·43	20·00	5·4	1·47	3·27	—
	X	1·28		0·73	3·08	3·81	—	—	2·86	—	5·81	—	•1·05	0·66	1·79	—	1·89	10·7	3·4	20·95	6·66	1·35	1·8	—
	XI	0·88		0·43	1·72	1·73	—	—	2·99	—	3·36	6·12	0·96	•3·42	2·43	—	4·11	9·84	3·13	13·59	3·27	1·20	0·75	—
	XII	1·19		0·76	2·80	1·94	—	—	3·11	—	2·22	6·35	•2·29	•2·77	2·51	—	4·64	10·00	3·09	23·63	5·66	1·33	0·88	—
1913	I	0·75		0·33	1·36	1·70	—	—	2·17	—	3·01	•8·41	•0·66	1·57	2·71	—	3·09	6·45	2·51	9·52	2·38	1·35	0·95	—
	II	0·68		0·5	—	3·02	—	—	1·91	—	2·26	•3·66	0·86	•2·21	1·37	—	2·03	9·80	2·50	7·84	2·43	0·93	1·04	—
	III	0·25		0	0·87	0·68	—	—	0·85	—	0·72	1·55	•0·83	•1·20	2·08	—	1·11	10·94	2·66	12·00	5·76	0·6	1·07	—
	IV	0·12		0·2	0·67	1·69	—	—	1·22	—	1·53	•1·77	•1·71	0·94	4·54	8·79	0·89	3·38	—	7·3	5·84	0·6	0	—
	V	0·36		0·1	1·69	1·33	—	—	1·20	—	—	•2·54	1·03	•1·74	5·24	3·57	0·97	2·57	—	—	—	0·68	—	35·54
	VI	0·36		0·21	0·46	0·75	—	—	1·06	—	—	3·57	•1·05	•2·92	5·91	2·37	1·15	9·33	—	—	—	0·9	—	—
	VII	0·69		0·64	1·94	0·99	2·46	—	2·41	—	—	•6·98	•1·23	0·88	5·93	1·72	0·85	12·73	—	—	—	1·03	—	—
	VIII	1·03		0·40	1·95	1·07	—	—	3·10	—	—	•6·82	0·85	0·65	6·48	3·14	1·43	8·85	—	—	—	1·59	—	—
	IX	0·73		0·31	3·66	2·58	•3·63	3·96	2·53	—	—	3·02	1·21	0·60	6·01	5·05	1·74	19·39	—	—	—	1·18	—	—
	X	1·50		0·2	5·61	2·64	2·51	•1·20	2·86	—	—	4·58	1·17	0·34	3·05	5·90	1·75	11·52	—	—	—	1·24	—	—

† (*a*) denotes Hokuto proper with a population of about 450 Japanese and Formosans;
(*b*) „ the neighbouring native village with a population of about 1,300.

Note.—The work recorded under columns 8 and 9 was stopped, and that recorded under 17 and 18 transferred to Industrial Bureau, under 19 and 20 to Taito Sugar Factory.
• Denotes so-called minor examination. Preliminary period: July, 1910—March, 1911. I Period: April, 1911—April, 1913. II Period: May, 1913— .

In general, the abrupt fall of the parasite index from the primary figure is remarkable, as the graphs show. In some places, where seasonal malaria yet prevails, however, some fluctuations are found in the curve. The difference of the results between these cases is due not only to mosquito-protection and medication, but also to social and local conditions. Malaria-splenomegaly, which is found spontaneously in endemic malaria districts (no Kala-azar being found in Formosa) diminishes *pari passu* with the advance of anti-malarial work.

B.—DECREASE OF FEVER-RATE

The malaria fever patients reported upon by the local practitioners, together with those who came to our stations for medical treatment and those discovered by police officials, give the *fever-rate* of the population. The following Table shows the fever-rate of some localities which adopted the anti-malarial measures.

TABLE IV.—Fever Rates of Localities (%).

		Chikutoki	Ensuiko	Ako	Banshiho	Hosan	Karenko	Yoshino	Bokusekikaku	Shinnun
1911	IV	—	—	—	—	—	5·67	—	4·23	—
	V	3·44	—	2·46	—	—	8·34	—	5·37	—
	VI	3·44	—	2·69	—	—	9·01	—	5·76	—
	VII	4·61	—	1·52	—	—	9·15	—	9·93	—
	VIII	7·48	—	1·31	—	—	7·69	—	8·15	—
	IX	5·80	—	0·97	—	—	7·38	—	5·33	—
	X	4·08	—	0·85	—	—	10·49	—	6·87	—
	XI	3·15	—	0·56	—	—	7·98	17·48	3·56	—
	XII	4·12	—	0·35	—	—	8·12	17·92	3·30	—
1912	I	3·34	—	0·65	—	—	5·24	5·69	2·25	—
	II	4·45	—	0·68	—	0·27	2·72	4·39	1·51	—
	III	6·74	—	0·54	—	0·23	1·78	2·93	1·63	—
	IV	4·34	—	0·69	—	0·13	1·69	2·94	1·87	—
	V	4·21	—	1·10	—	0·32	5·30	5·63	1·81	—
	VI	1·62	—	1·29	—	0·37	4·91	4·10	2·63	12·90
	VII	9·21	—	0·65	5·80	0·3	5·0	4·93	2·80	7·80
	VIII	5·36	—	0·52	3·05	0·26	2·72	6·16	3·70	9·38
	IX	3·89	—	0·5	2·12	0·26	2·84	13·06	1·83	3·08
	X	3·36	—	0·4	0·33	0·19	4·68	10·98	2·07	12·59
	XI	3·26	3·49	0·7	1·87	0·07	8·38	16·00	2·65	11·59
	XII	3·16	0·53	0·85	2·98	0·16	5·00	9·13	2·32	7·20
1913	I	2·42	0·32	0·7	2·03	0·09	4·34	5·62	1·52	5·69
	II	2·85	0·12	0·43	1·18	0·09	2·46	6·40	1·28	4·27
	III	2·65	0·14	0·5	1·43	0·01	1·59	4·0	0·97	0·95
	IV	5·30	0·08	1·0	1·91	0·01	1·94	—	0·62	1·00

The fever-rate recorded in the localities decreases approximately parallel to that of the parasite index.

Owing to the seasonal prevalence of malaria, there are sometimes observed fluctuations in the curve of the fever-rate, e.g., it sometimes precedes that of the parasite index curve. In some localities, for instance, Rigyobi, where medical organisation is still in an imperfect state, the fever-rate is small, while the parasite indices are large. Among the Formosans the fever-rate is low and the parasite index high, whereas the contrary is the case with Japanese. The parasite index and the fever-rate taken together make up the malaria-rate of the locality.

TABLE V.—Malaria Rate of Karenko (abstracted).

		Japanese			Formosans			Total Malaria-rate %
		Parasite-index %	Fever-rate %	Malaria-rate %	Parasite-index %	Fever-rate %	Malaria-rate %	
1911	VII	2·86	12·34	15·20	4·78	6·62	11·40	12·54
1912	I	4·85	7·27	12·12	6·50	3·14	9·64	10·90
1912	VII	2·93	7·26	10·19	3·29	2·97	6·26	9·07
1913	I	2·38	6·33	8·71	3·93	2·02	5·95	7·43

C.—DECREASE OF MALARIA DEATH-RATE

Now that the anti-malarial work has made favourable progress, the malaria death-rate has markedly diminished. In fact, in some well-known malaria districts, such as Bokusekikaku, no malaria deaths have been reported in the last two years.

In the Eastern part of Formosa, the malaria-perniciosity is exceedingly high, and consequently malaria deaths are of frequent occurrence, chiefly due to blackwater fever. In other parts, deaths due to malaria occur rather infrequently.

As above mentioned, in the course of the anti-malarial operations, the malaria endemicity, prevalence, and death-rate have apparently diminished in general. Although in the early stage of the campaign the people disliked taking quinine on account of its bitterness, and tried to shun the strict administration of it, yet afterwards they became convinced of its merit, and felt much indebted to the systematic treatment for their recovery from the chronic disease.

TABLE VI.—Death-rate, and Malaria Death-rate (°/₀₀) in localities where the Anti-malarial work was carried on for over two years.

		Chikutōki	Akō	Karenkō	Bokusekikaku
Preceding year	Population	1,800	5,000	2,500	991
	Deaths	109	169	96	34
	Death-rate	60·56	33·80	38·40	34·31
	Malaria deaths	13	25	16	15
	Malaria death-rate	7·22	5·00	6·40	15·14
First 12 months	Average Population	1,870	5,400	2,836	1,001
	Deaths	90	190	82	28
	Death-rate	48·13	35·19	28·91	27·97
	Malaria deaths	2	14	15	0
	Malaria death-rate	1·07	2·59	5·29	0
Second 12 months	Average Population	1,652	5,997	3,537	1,215
	Deaths	76	188	91	29
	Death-rate	46·00	31·35	25·73	23·87
	Malaria deaths	1	11	6	0
	Malaria death-rate	0·61	1·83	1·70	0

TABLE VII.—Malaria perniciosity in Formosa.

Date	Part	Locality	Total number of plasmodia carriers	Of which, number of malignant tertian	Perniciosity %	Author
1910	N.	Hokuto	162	73	45·00	Hatori
1910	W.	Inrin	716	159	22·20	Nakajima
1911-2	W.	Chikutoki	488	172	25·13	Mori
1911-3	S.	Ako	1,080	401	37·10	Kojima
1911-3	S.	Hosan	1,656	598	36·10	Kojima
1911-3	E.	Karenko	2,625	1,871	71·28	Hatori
1911-3	E.	Yoshino	549	301	54·80	Hatori
1911-3	E.	Bokusekikaku	345	238	69·00	Hatori

IV. SOME DATA ON QUININE TREATMENT AND THE EXPENSES INCURRED

The total number of persons treated by us with quinine provided by the Government was 14,454. Among them, there were some persons who moved to other districts during the course of the treatment. Some suffered from malaria many times, not only owing to relapses, but also owing to reinfection. Some who have previously or suddenly acquired the haemolytic disposition as the result of chronic infection, are often attacked by blackwater-fever at the beginning or during the course of quinine treatment. Notwithstanding our utmost precautions, this unfavourable complication is frequently met with on the Eastern coast, though rather rarely elsewhere. Other quinine idiosyncrasies were only rarely encountered.

The quinine administered to 14,454 men at the expense of the Government amounted to the following:—

	Kgr.		Yen	Sen
Quinine hydrochlor. (in tablet)	211·930	(per 1 lb.	9	25)[*]
Euchinin	37·270	(per 1 lb.	15	82)
Esanopheles pills	333,000	(per 1,000	1	90)
Total cost	6,271 Yen 37 Sen.			

As may be seen from the above, the State Quinine used by us cost approximately 0·434 Yen (=*ca.* 11d.) per man treated. The fund of the 'Bōeki-kumiai'[†] is chiefly used for town-cleansing, minor works, providing needy people with mosquito nets, &c.

V. THE ORDINANCE AND REGULATIONS WITH REFERENCE TO MALARIA PREVENTION

As mentioned above, the anti-malarial operations have met with a successful result in the localities where the measure was adopted. The Government have accordingly decided upon the plan of extending the field of warfare over other principal malarious localities. Realising, however, that it was more economical and advantageous to transfer the work to the local governments, in April last, Ordinance and Regulations respecting Malaria prevention were announced, and put in force in the following month of May.

---

[*] Yen = 2s. 1d. Sen = ¼d. [†] See page 538.

REGULATIONS FOR THE PREVENTION OF MALARIA

On April 10th, 1913, an ordinance was passed for the prevention of malaria in Formosa, and under this ordinance the following regulations were issued:—

Art. 1. The powers vested in the local administrative offices with reference to the malaria prevention regulations shall be exercised by the local governors.

Art. 2. The local governor may at any time put in force anti-malarial measures, if he deems it necessary. In that case, sanction must previously be obtained from the Governor-General as to the area, methods and length of time.

Art. 3. If the Governor-General finds it necessary, he may at any time give orders to the local governors to carry out malaria prevention measures, in accordance with the prescribed area, methods and period of time.

Art. 4. The local governors are required to give notice with reference to the area and period of time decided upon.

Art. 5. The inhabitants and sojourners within the area, where the malaria prevention measures are to be taken, have to comply with the regulations concerning physical examination, medical treatment and administration of quinine, and can on no account decline the summons issued by the authorities for such purposes.

Art. 6. The inhabitants and sojourners within the area, where the malaria prevention measures are to be taken, shall use mosquito nets in their beds, in compliance with the instructions of the governors.

Art. 7. The occupiers or owners of land-property and buildings within the area, where the malaria prevention measures are to be taken, shall maintain cleanliness in their land-property and buildings in obedience to the directions given by the authorities concerned.

Art. 8. The said occupiers or owners shall carry out cleaning in their land-property and buildings, in accordance with the regulations laid down by the governors.

Art. 9. The epidemic disease prevention corporations (Bōeki-kumiai) and the self-governing associations (Ho-kō) shall, in compliance with the directions of the proper authorities keep clean the public roads, drains, parks, and other public places, and shall remove filth and sweepings collected by the people.

Art, 10. In case these two bodies should undertake the duty of removing the filth, the epidemic disease prevention associations shall come first and then the self-governing associations.

Art. 11. The local governors may order the above-mentioned bodies to make provisions regarding malaria prevention, and cause them to be carried into operation.

Art. 12. The medical practitioners holding their offices within the malaria prevention area shall report to the neighbouring police stations whenever they have discovered malaria cases.

Art. 13. The local governors may, with the permission of the Governor-General, collect the expenses incurred from the persons who have received medical treatment and taken medicines, in accordance with Art. 5.

Art. 14. The heads of the government offices, existing within the area where the malaria prevention measures are to be taken, shall consult with the governor concerned, as to the due measures to be taken in their own offices.

Art. 15. The local governor may order the members of the Epidemic Diseases Prevention Corporations, and leading heads of the village communities, to engage in the malaria prevention work.

Art. 16. Those who have infringed Arts. 5, 6, 7, 8, and Art. 12 shall be liable to be fined.

ADDITIONAL RULES

Art. 1. The local governors shall, at stated periods of time, submit reports to the Governor-General, giving particulars as to how the preventive measures have been undertaken and the result obtained.

Art. 2. The maintenance of cleanliness, as designated by the police officials and the sanitary officers, shall be directed towards preventing the breeding and the living of mosquitos.

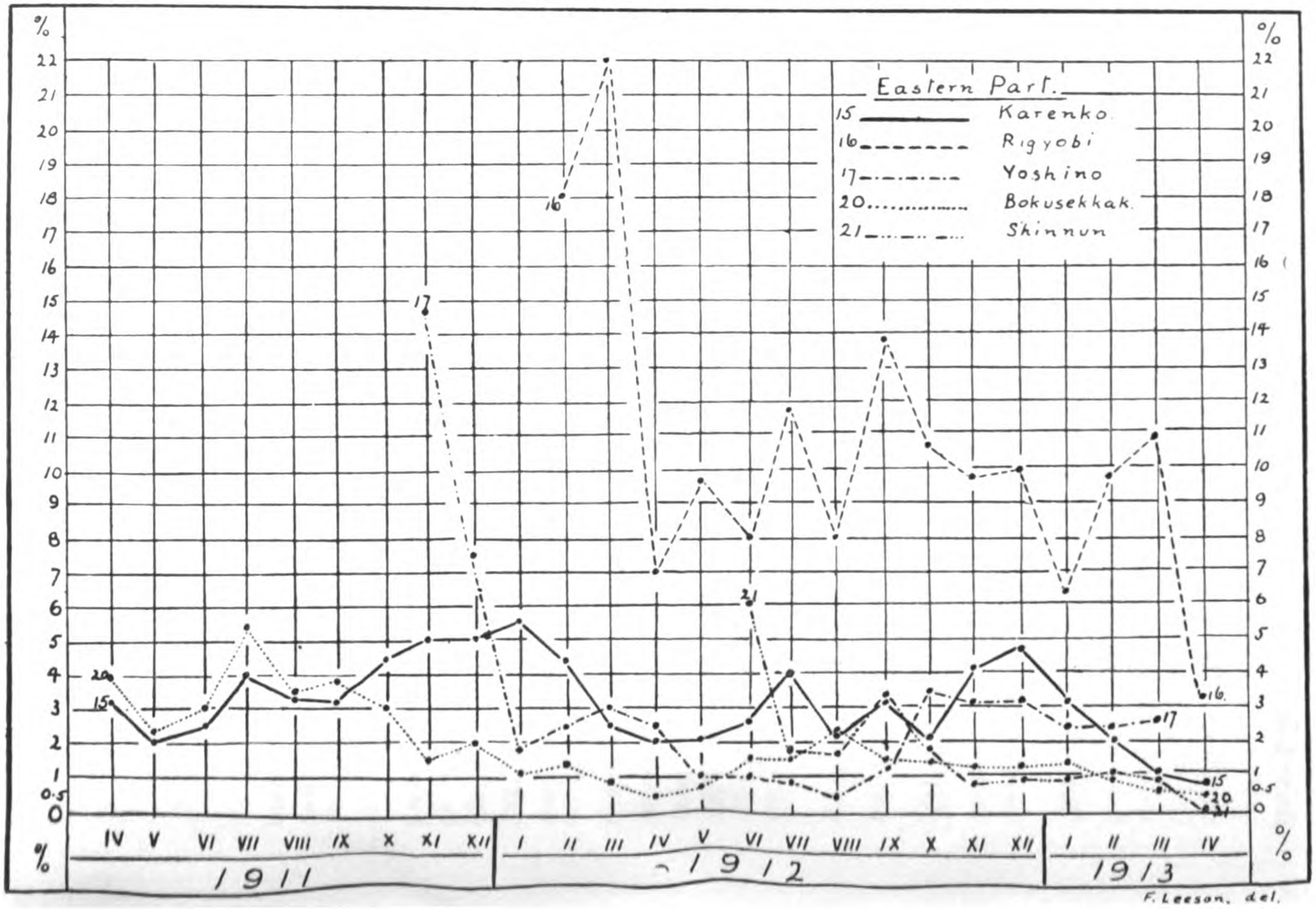
Art. 3. If he finds it necessary in order to prevent malaria, the local governor shall, in accordance with Art. 7 of the malaria prevention regulations, issue instructions to the inhabitants to carry out thorough cleansing in the specified ways.

Hereupon, the duties of the campaign have been taken over by the local governments, who are to carry out the regular work and to send in monthly reports on the result obtained. The expenses incurred for purpose of examination and medical treatment are disbursed from the State Treasury.

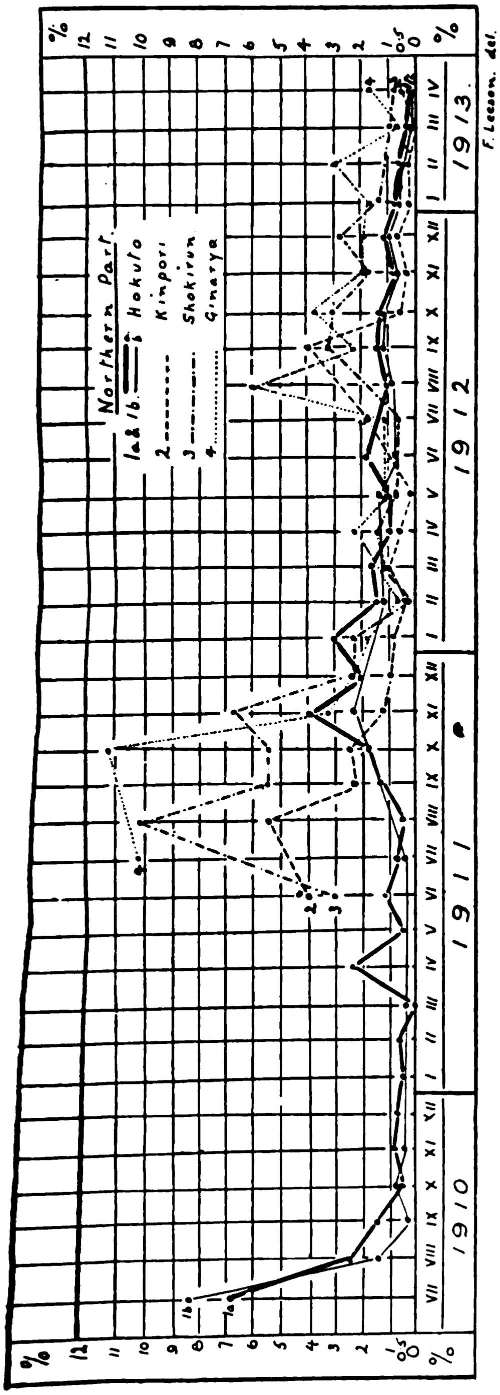
As above alluded to, anti-malarial operations will, in accordance with the said Ordinance, be hereafter carried out on a wider scale over various localities of the island. It may, therefore, be not many years before malaria-free and better health conditions will be secured, according as local sanitation improves, in the localities hitherto known as malaria-stricken.

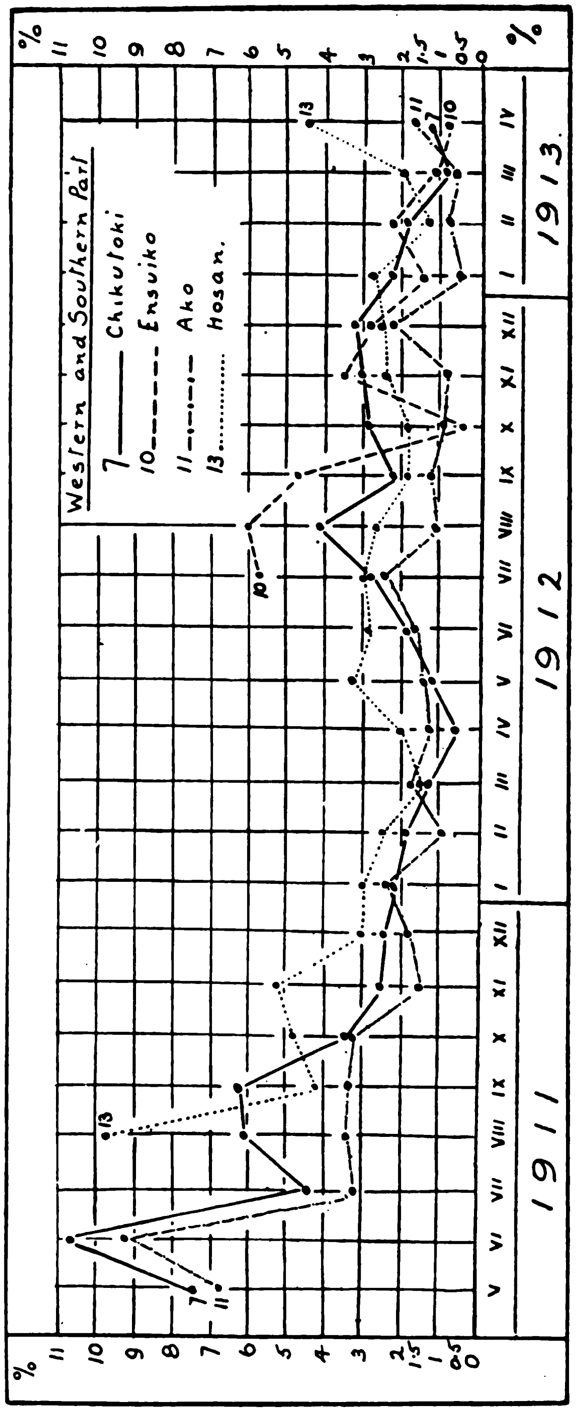
In conclusion, I wish to express my grateful thanks for the very valuable suggestions I have received from Prof. Dr. T. Takaki in preparing the above report.

Graphs showing the monthly change of Parasite Indices in some localities, Formosa.



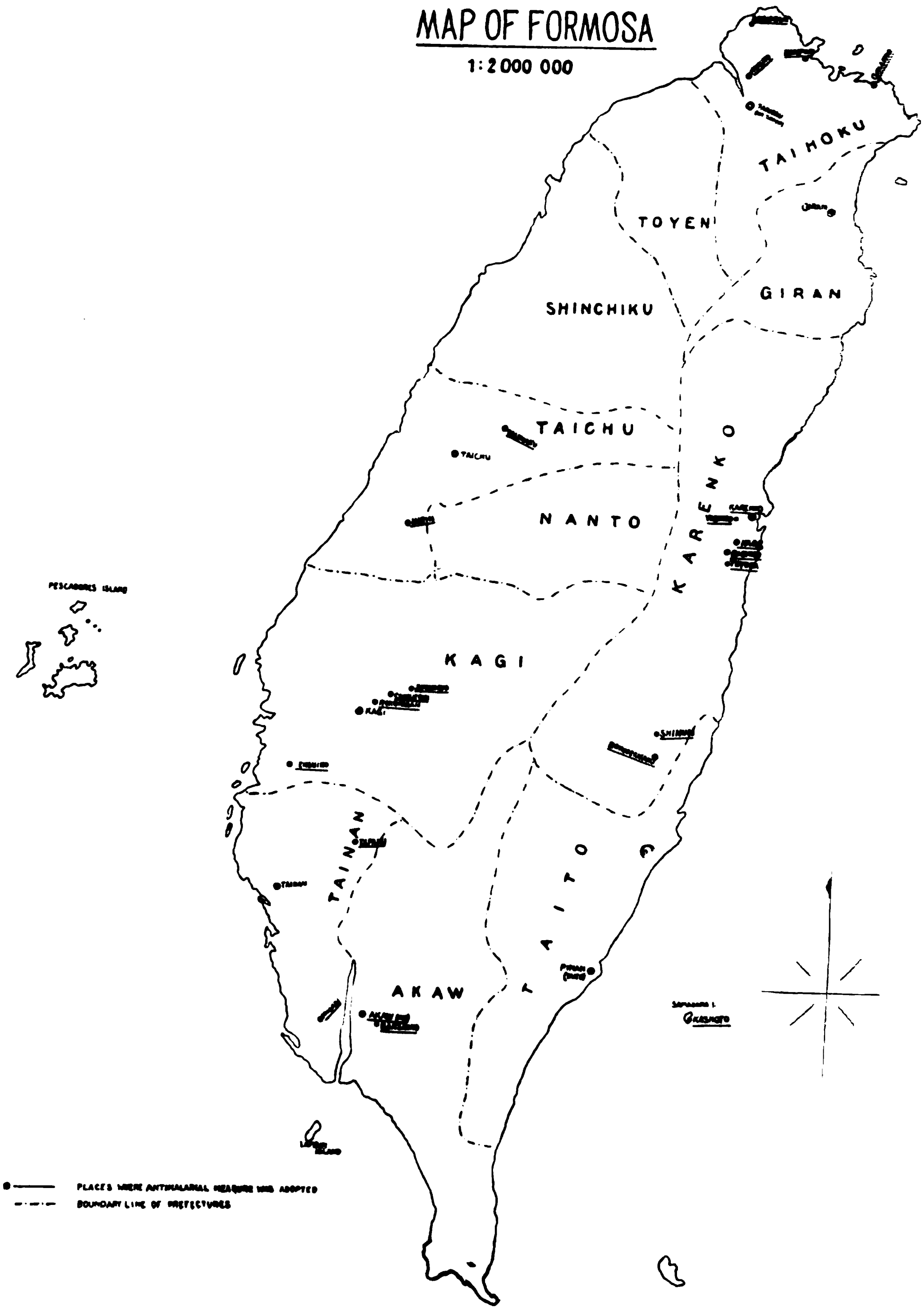
F. Leeson, del.


Northern Part.
1a & 1b Hokuto
2 Kinpori
3 Shokiryu
4 Ginarya
%
12
11
10
9
8
7
6
5
4
3
2
1
0.5
0
VII VIII IX X XI XII
1910
I II III IV V VI VII VIII IX X XI XII
1911
I II III IV V VI VII VIII IX X XI XII
1912
I II III IV
1913
F. Leeson. del.


Western and Southern Part
7 Chikutoki
10 Ensuiko
11 Ako
13 Hosan.
%
11
10
9
8
7
6
5
4
3
2
1.5
1
0.5
0
V VI VII VIII IX X XI XII
1911
I II III IV V VI VII VIII IX X XI XII
1912
I II III IV
1913
F. Leeson, del.

MAP OF FORMOSA

1:2000 000



KLOSSIELLA SP. IN THE KIDNEY OF A GUINEA-PIG

BY

HARALD SEIDELIN, M.D.

(*Received for publication 7 August*, 1914)

PLATES XXVIII, XXIX

A parasite of the renal epithelia of mice was described by Smith (1889), and was later on studied in detail by Smith and Johnson (1902), who named it *Klossiella muris*. No further cases have been published, as far as I am aware, and the life-history of the parasite is not known in all its phases. The present case of a similar nature could not be fully investigated, as the parasite was found casually during the routine examination for another purpose of guinea-pigs' organs which had already been embedded in paraffin. The available material was, however, quite sufficient to show that the parasite here described possesses so great a resemblance to that of Smith and Johnson that both may be regarded as belonging to one and the same genus, whilst the question of their specific identity or otherwise cannot at the present time be finally decided.

The parasite was observed in the kidney of a guinea-pig (No. 306) which had been used for yellow fever experiments at the Medical Research Institute, at Yaba, near Lagos, Nigeria. The animal was found dead five days after it had been infected by means of mosquito transmission. It had been purchased locally. The lesions encountered at the post-mortem examination are of no interest in this connection except the presence of albumin in the urine recovered from the bladder, but as albuminuria was a very common occurrence in the yellow fever infected guinea-pigs, also this phenomenon is probably of no special importance in this case. The macroscopical examination of the kidneys showed no abnormal conditions; microscopically there were marked necrobiotic changes of the epithelia, especially in the convoluted tubules, also in such that were not infected with *Klossiella*, and many tubules, as well as some Bowman's capsules, contained granular exudates. There were also some irregularly distributed patches of hyperaemia. On the whole, and leaving out of consideration the phenomena directly attributable to the presence of the parasites, the anatomical changes were similar to those seen in many of the animals infected with yellow fever. The urine was not subjected to a microscopical examination.

In the kidney the coccidium-like parasite was observed in the first section examined; subsequently numerous sections were prepared, and parasites were more or less abundant in all. According to my observations, the parasite does not appear to be a common one in West African guinea-pigs, as this was the only case found amongst about twenty guinea-pigs of the Yaba series, the organs of which were examined in Liverpool; no special investigation was made with regard to this question, but obviously such a comparatively large parasite could not have escaped observation in the sections examined. I have found no similar case in guinea-pigs experimented upon in Liverpool.

The sections were stained with haemalum or acid haemalum and eosin, or with iron-haematein,[*] without counter-stain or counter-stained with Hansen's aceto-picro-acid fuchsin solution.

The earliest stage in the evolution of the parasite is apparently the form shown in Plate XXVIII, fig. 1, consisting of an approximately globular body enclosed in a vacuole in the protoplasm of an epithelial cell in the cortex. The diameter of the parasite in this stage is just over 2 μ, its protoplasm is very scarce and surrounds an irregularly shaped nucleus. The following stages show a gradual increase of size, especially of the protoplasm, so that the parasite, before division sets in, measures about 6 μ in diameter. The nucleus now begins to divide into two, four, and more daughter nuclei, which from the start, but especially later on, occupy the peripheral portions of the parasite's body. The protoplasm condenses around the daughter nuclei, and daughter elements are formed at the periphery of the mother organism, apparently by a sort of budding process, a restiform body being left behind in the central portion. It is, however, exceptional to observe the existence of a well-marked peduncle; as a rule the daughter parasites appear quite separated from the mother organism or restiform body, but in a few instances some kind of connection with the central mass is undoubtedly present, and this observation confirms the existence of a budding process as described by Smith and Johnson. The rarity of a visible connection may depend on a short duration of this stage of evolution, or upon difficulties of observation in the fixed specimens. The number of daughter elements is also difficult to determine, as the comparatively large size of the parasites usually makes it impossible to observe the whole of a distended cell with its contents in one section, and conclusive evidence as regards the number of elements could hardly be obtained from the examination of serial sections without reconstruction by means of wax models, a procedure which has not been attempted. As far as can be determined, the number of elements resulting from this division varies from sixteen to twenty, eighteen being the number most frequently met with in organisms which appear complete. The daughter organisms remain—at any rate this appears to be the rule—inside the same host cell, where they undergo a further division which results in the formation of a large number of very small, more or less fusiform elements. These small bodies are so closely packed together inside a common membrane that their total number is difficult to determine, but it appears to be about thirty when the division has been completed.

---

[*] After further experimentation, I now recommend the following two solutions for the iron-haematein stain :—

Solution A.—1 gm. of haematein dissolved by heating in 100 c.c. of 90 % alcohol. If necessary, filter before use.

Solution B.—Pure hydrochloric acid 1 ; iron perchloride (pure) 2 ; aq. dest. 100.

Otherwise as described in *Parasitology*, Cambridge, July, 1911, IV, 2, pp. 94-103.

The whole evolution here described is shown in the figures 4-15. The diameter of the full-grown mother organism, before any division of the nucleus has taken place, is, as stated above, about 6 μ; with the increasing number of nuclei the size of the parasite also increases, and just prior to the separation of the daughter organisms it measures about 17 μ by 12 μ. The cysts containing daughter organisms are considerably larger—in fig. 11 the longitudinal diameter is 31 μ and the transverse diameter 22 μ—but none has been measured containing a full number of completely developed daughter organisms. These elements measure about 10 μ by 6 μ, and the fusiform bodies about 8 μ by 1·5 μ.

Before the question of the ontogenetic importance of these various elements is considered, it may be of value to discuss their relations to their host-cells. Smith and Johnson state that in the fresh specimens a vacuole was observed surrounding the parasite, but that this vacuole was usually not visible in sections. In all my specimens a vacuole was plainly visible, and as a rule very marked. This difference can hardly be due exclusively to the different techniques employed. Smith and Johnson fixed their material in Zenker's fluid, whilst I used Schaudinn's sublimate-alcohol. They ascribe the obliteration of the vacuole to shrinkage, but the method of fixation employed by myself usually produces an even more marked shrinkage than Zenker's fluid. For this reason their explanation can perhaps not be accepted unconditionally, but the interesting fact remains that vacuoles have been observed in both cases, and it is possible that the shrinkage may have been more uniform in my material, Schaudinn's fluid perhaps affecting the tissues of the host-cells and of the parasites more equally than Zenker's. As long as vacuole and parasite are comparatively small, no changes of any importance are seen in the host-cell, but the growth of the parasite and correspondingly increasing size of the vacuole cause an enormous distension of the cell, the protoplasm of which becomes more dense and homogeneous than is normally the case in the renal cells; also nuclear changes are seen, mainly loss of structural details and of staining properties. Pycnotic nuclei are rarely seen. Half-grown daughter parasites are often seen about eighteen together in a cell in which there is no evidence of rupture, but full-grown individuals of the same stage have never been seen in such large numbers under similar conditions. It is possible that this difference may be apparent only, and the explanation may be that in the latter case the excessive distension of the cell takes place in various directions, following also the longitudinal axis of the tubule, but this explanation is not supported by the examination of serial sections. It appears more probable that the number of individuals has at this time really become reduced, either because the parasites, having reached this stage, successively leave their host-cell, or because a considerable portion of their number die off, leaving comparatively few to complete their division. The emigration theory appears the most plausible one. Another interesting detail observed at this stage is that the fully, or almost fully, divided daughter organisms do not appear all together in one large vacuole, but each one has its own more or less well defined vacuole, separated from its neighbours by strands of protoplasm.

These relations of the parasites to the epithelial cells are illustrated in the drawings, figs. 1-3 and fig. 12, and in the microphotographs, figs. 23, 25, and 26. The cells mostly affected are the epithelia of the convoluted tubules of the first order, but also those of the second order and the straight tubules in the loops of Henle are sometimes the seat of parasites. The collecting tubules have not been seen infected, nor have parasites been seen in epithelial cells of the renal pelvis, but in the latter place some few cells had a very striking appearance, which suggested that they might possibly have contained parasites which had escaped before the time of examination. Such a cell containing a large vacuole, empty except for a small quantity of granular débris, is shown in fig. 21.

The observations here described are, of course, quite insufficient to establish the life-cycle of the parasite, but in the light of the investigations of Smith and Johnson and of the existing knowledge of similar parasites, it is possible to discuss the mutual relationship between the various elements observed, although much must necessarily remain hypothetical until an opportunity is found for examination of fresh material and until successful transmission experiments are carried out.

Smith and Johnson are of opinion that the evolution which takes place in the renal epithelia, as described, represents the sporogonic part of the parasite's life-cycle. The sporont divides into spores, which again subdivide into sporozoites. Intact spores having been found in the bladder, the authors believe that these elements escape from the body of the host with the urine, and that the sporozoites do not become free until the spores have been ingested, with food or water, by a new host. No gametogony was, however, observed and the schizogonic cycle was not found, unless some elements found in the glomerulus represent this stage, a question which the authors leave open. Doflein (1911), however, points out that the so-called sporonts of *Klossiella muris* closely resemble the agamonts of *Caryotropha mesnili*, and both Lühe (1906) and Minchin (1912) are apparently of the same opinion, since they include *Klossiella* in the family of Caryotrophidae. The latter view is supported by several circumstances observed in my material and presently to be mentioned; it appears to me to be the correct one, but no conclusive proof can at present be given, and it ought to be mentioned as a further possibility that the question may be still more complicated than has hitherto been suggested. It is, in fact, quite possible that both agamonts and gamonts may be represented in the forms observed in the renal epithelia.

Returning to my own material, I have found no special glomerular forms of the parasite, but have only once seen an individual in one of the parietal cells of a Bowman's capsule, an individual which appeared identical with the forms represented in fig. 9. I can therefore limit the discussion of my case to the elements observed in the tubular epithelia. The convoluted epithelia were chiefly affected, and the occasional presence of parasites in other tubules seems to be a factor of no ontogenetic importance, as no forms were found in the straight tubules or in the second convoluted tubules which had not already been observed in the convoluted tubules of the first order. It can hardly be doubted that the figures 4-10 represent stages of an uninterrupted evolution, and it is exceedingly probable that the stage shown in fig. 11 follows directly after that in fig. 10, but some doubt arises with regard to the stage shown in figs. 12 and 26. These forms are fairly common; they differ, as has been pointed out already, in two respects from the previous stages: the number of elements is comparatively small, and each element appears enclosed in a separate compartment instead of being all contained in one and the same vacuole of the host-cell. Various explanations of these differences were attempted above, but no one was quite satisfactory, for which reason the hypothesis appears justifiable that some intermediary, as yet unknown, process has taken place here, possibly in the nature of a copulation. If this be the case, the stage shown in fig. 3 may be intermediary between those of fig. 11 and fig. 12, and further support is lent to this hypothesis by the existence of forms like that shown in the micro-photograph fig. 27. Such forms are rare, but several have been found, their chief characteristic being that a number of small elements belonging to two different types (shown in figs. 17 and 18) are present in one large cavity. Thus, an anisogamic process is suggested, which may possibly take place inside the same cavity, and figs. 3 and 12 may represent the resulting sporonts. In this case the bodies shown in figs. 15 and 16 would be different from those depicted in figs. 17, 18, and 19, the former being sporozoites and the latter gametes, or gametocytes. Should the view expressed by Doflein really be the correct one, then all these small bodies would be sexual forms, but the place and character of the copulation process would be entirely unknown. As an argument against this theory may be quoted the observation made by Smith and Johnson that intact 'spores' are found in the bladder. The urine was not examined microscopically in the case of my guinea-pig, but I never saw these bodies intact in the renal pelvis in sections; on the other hand, I observed a number of bodies which in all probability are empty membranes, from which the 'fusiform' or 'falciform' bodies have escaped, but none of the latter were seen.

In conclusion, it appears probable that the first process of division is a schizogony, whilst it is uncertain whether the second division represents a schizogonic sub-division or a sporogony, of which some stages remain imperfectly known. Either the fusiform bodies or their immediate progeny are probably responsible for the transmission; Smith and Johnson attempted experimental transmission by the feeding of animals with kidneys containing the secondary division stages, but obtained no conclusive results.

It may here be mentioned that Stevenson, according to private information to Minchin (1912), considers it possible that the parasite described as *Klossiella muris* may represent an evolutional stage of *Haemogregarina musculi*. Smith and Johnson do not mention the presence of blood parasites in their mice; in my guinea-pig *Paraplasma flavigenum* was the only blood parasite observed.

Several particulars suggest that the parasite here described may represent another species than *Klossiella muris*. Its dimensions, as measured in sections, are as a rule smaller, but as Smith and Johnson measured on fresh material, the measurements cannot be directly compared. The number of elements resulting from the second division is given as twelve to fourteen in *Klossiella muris*, whilst eighteen appears to be the average number in my case, a difference which is not easily explained by mistaken observation, though an appearance like that of fig. 11 in Smith and Johnson's paper suggests that some individuals at the bottom or top might have been missed out from the drawing, as otherwise the budding process would appear to take place from a disc and not from a globular body, as was undoubtedly the case in my observation. The numbers resulting from the second division were essentially identical. The presence of spherical plastin granules in the mother organism is mentioned by Smith and Johnson amongst the specific characters; the parasites seen in my sections contained a large number of highly refracting granules, but as similar, though smaller and less numerous, granules were also present in non-infected cells, it is hardly safe to discuss their nature and importance. The occurrence of the parasites in different animals and in different localities (Smith and Johnson's in Massachusetts, mine in Nigeria) cannot be regarded as specific differences. Nor does it appear that the localisation of some parasites in the glomeruli in Smith and Johnson's cases constitutes a material difference from mine, as the glomerular forms depicted by them appear identical with some forms in the convoluted tubules in my case.

Of all the differences mentioned, importance can only be attached to the different number of daughter organisms. This makes it probable that the parasite here described represents a new species, for which the name of *Klossiella cobayae* is proposed.

Addendum: Since the above was written, I have observed identical parasites in sections of a kidney of another guinea-pig from the same locality.

REFERENCES

DOFLEIN, F. (1911). Lehrbuch der Protozoenkunde. 3rd ed. Jena.

LÜHE, M. (1906). Die im Blute schmarotzenden Protozoen und ihre nächsten Verwandten. In Mense's 'Handbuch der Tropenkrankheiten,' III, Leipzig.

MINCHIN, E. A. (1912). An introduction to the Study of the Protozoa. London.

SMITH, T. (1889). In *Journ. Comp. Med. Surg.*, X, p. 211, (not accessible).

SMITH, T. and JOHNSON, H. P. (1902). On a coccidium (***Klossiella muris***, gen. et spec. nov.) parasitic in the renal epithelium of the mouse. *Journ. Exp. Med.*, Baltimore, VI, 3, pp. 303-316.

EXPLANATION OF PLATES.

PLATE XXVIII

The figures have been drawn by means of Abbé's camera lucida, at a magnification of about 1350 lin.; Zeiss' apochr. imm. obj. 3 mm., comp. oc. 12.

H-E means Acid haemalum and eosin, I-H Iron-haematein and Hansen's solution.

Fig. 1. Young stage of *Klossiella cobayae* in epithelial cell of convoluted tubule. H-E.

Fig. 2. Somewhat more advanced stage; convoluted tubule. H-E.

Fig. 3. Primary division completed, secondary division beginning; convoluted tubule. H-E.

Figs. 4-10. Stages of primary division, all from epithelial cells in convoluted tubules. Fig. 4 I-H, all others H-E.

Fig. 11. Secondary division beginning, convoluted tubule. I-H.

Fig. 12. Secondary division completed; convoluted tubule of second order. I-H.

Fig. 13. Dividing daughter parasite; convoluted tubule. I-H.

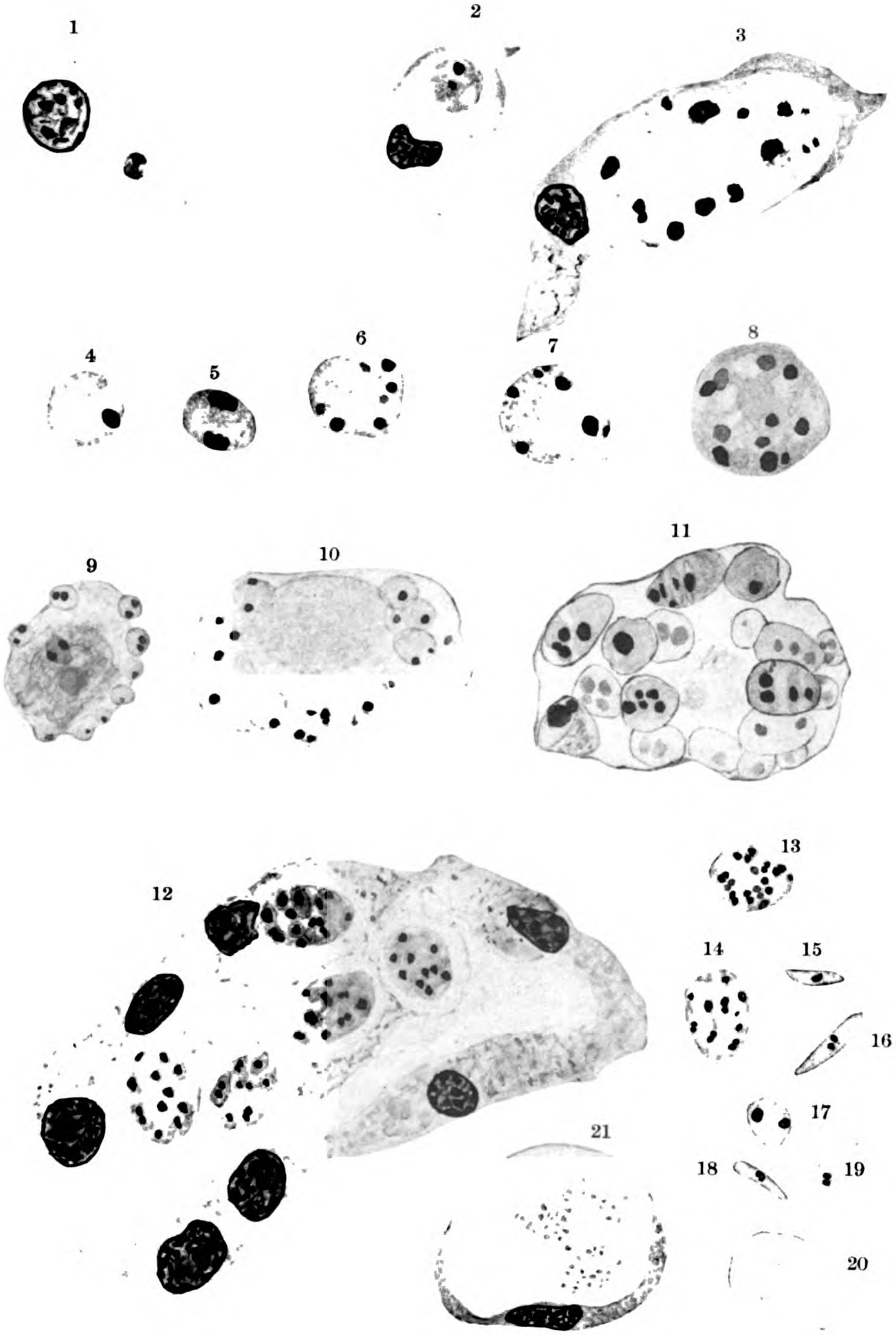
Fig. 14. Completely divided daughter parasite; convoluted tubule. I-H.

Figs. 15-19. Elements formed by secondary division, 15 and 16 from convoluted tubule, 17, 18, and 19 from ascending limb of Henle's loop, the former I-H, the latter H-E.

Fig. 20. Empty membrane from cavity of renal pelvis. H-E.

Fig. 21. Vacuole-containing cell from epithelium of renal pelvis. H-E.

PLATE XXVIII



Harald Seidelin del.

Plate XXIX

Micro-photographs; apochr. imm. obj. 3 mm., comp. oc. 8 or 12.

Fig. 22. Heavily infected straight tubule, apparently ascending limb of Henle's loop. H-E. × 630.

Fig. 23. Young parasites in convoluted tubule. I-H. × 1250.

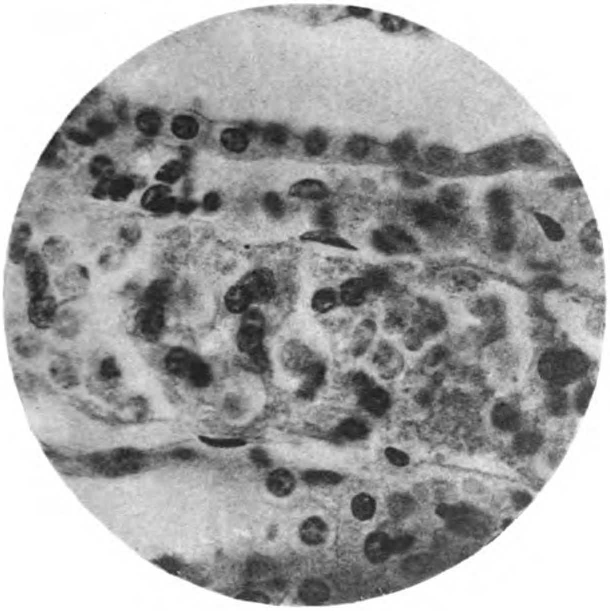
Fig. 24. Primary division, convoluted tubule. H-E. × 630.

Fig. 25. Beginning secondary division, convoluted tubule. I-H. × 1250.

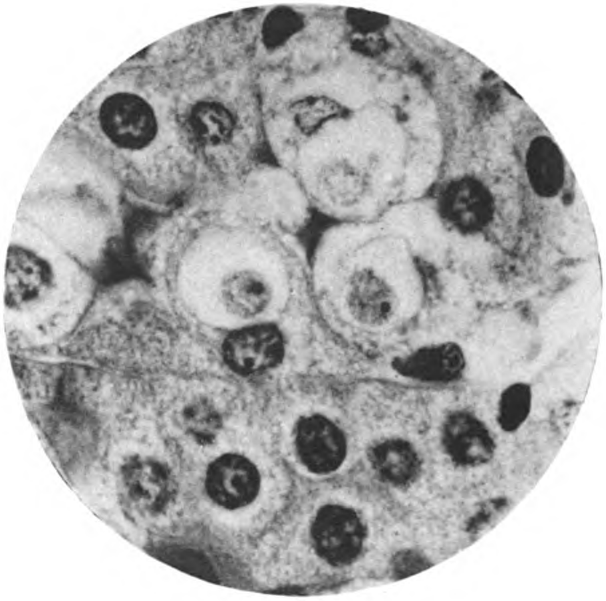
Fig. 26. Secondary division, convoluted tubule. I-H. × 1250.

Fig. 27. Elements formed by secondary division, ascending limb of Henle's loop. Iron-haematein. × 1250.

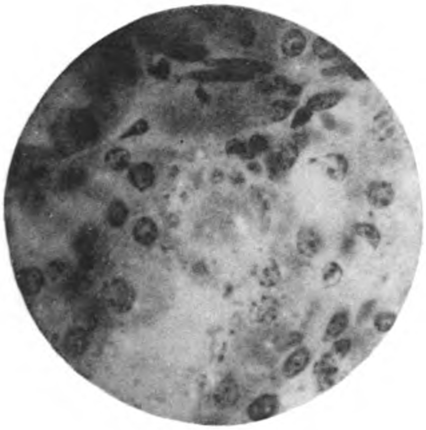
22



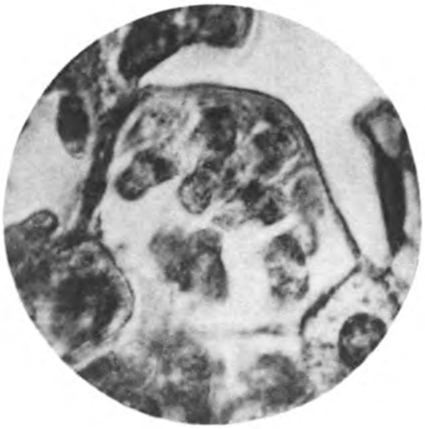
23



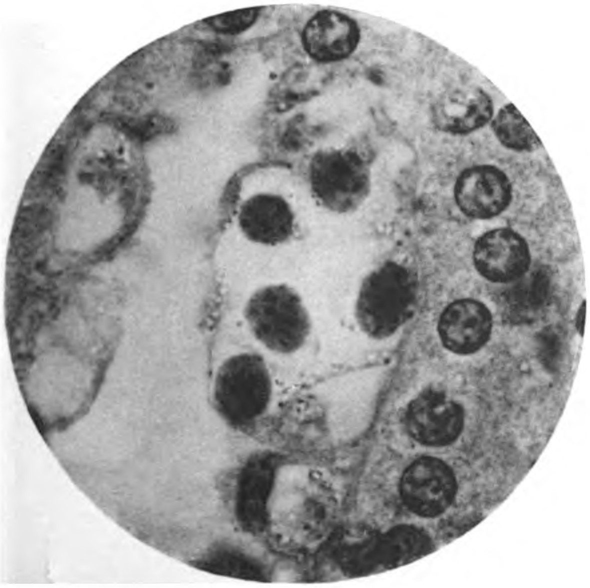
24



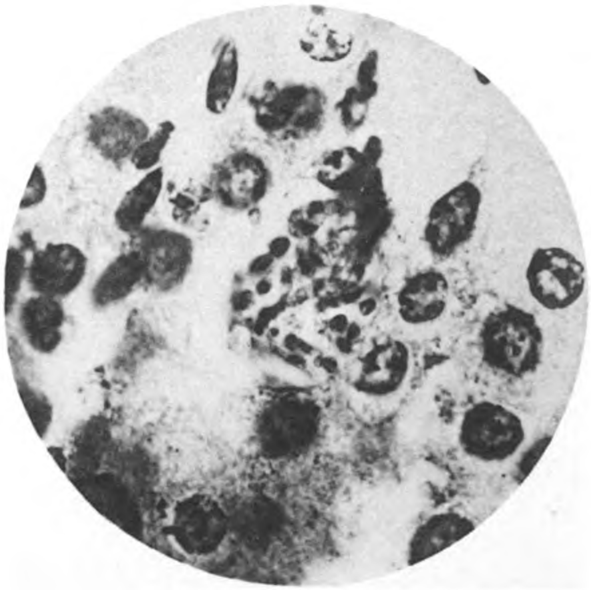
25



26



27



CHANGES IN THE NEUTROPHILE BLOOD PICTURE OF ARNETH OBSERVED IN CHILDREN LIVING IN TROPICAL QUEENSLAND

BY

A. BREINL

AND

H. PRIESTLEY

FROM THE AUSTRALIAN INSTITUTE OF TROPICAL MEDICINE, TOWNSVILLE

(*Received for publication* 28 *August*, 1914)

ONE CHART

One of the most important practical advances in haematology within recent years was the discovery by Arneth of the definite relationship in the number of neutrophile leucocytes containing a different number of nuclei or nuclear fragments.

In 1904 Arneth published a monograph pointing out, as the result of prolonged and painstaking researches, that during the course of certain infectious diseases the nuclear conditions of the neutrophile leucocytes vary in a definite direction.

Arneth divided the neutrophile leucocytes into five main classes, according to the number of nuclei or nuclear fragments they contained, and each class into a number of sub-classes.

His first class contained three sub-classes, according to the shape of the nucleus, being either sickle-shaped (W), baton-shaped (T), or resembling that of a typical myelocyte (M).

The other four classes, with two to five or more nuclear fragments, were sub-divided again in accordance with the shape of the fragments which are ribbon-shaped or round. The number of ribbon-like nuclei were tabulated under S (Schlinge), and the round nuclear fragments under K (Kern).

The following Table (1), quoted from his monograph, may serve as a typical example:

Table 1.—Dr. A.

1			2			3				4					5 and more		
M	W	T	2K	2S	1K 1S	3K	3S	2K 1S	2S 1K	4K	4S	3K 1S	3S 1K	2K 2S	5K	4K 1S	3S 2K
—	—	4	—	17	4	2	13	14	19	4	—	7	4	8	2	1	1

Arneth counted one hundred successive neutrophile leucocytes, and tabulated the results. His observations indicate that the changes in the number of leucocytes of classes I and II were most important, and devised, as a convenient means for comparing the results in different cases, his index which is the sum of these classes. Bushnell and Treuholz (1908) found it advisable to add half the number in class III.

Arneth's original observations were made on blood films stained with Ehrlich's triacid stain. Later observers used Giemsa's stain with satisfactory results.

The average normal counts as given by Arneth (1904), Paulicek and Simon for adults, and by Esser (1906) and Orland (1907) for infants, are compiled in Table 2.

Table 2

	Class I	Class II	Class III	Class IV	Class V	
Arneth ...	5	35	41	17	2	Adults
Paulicek ...	4	28	52	14	2	„
Simon	4 to 9	21 to 47	33 to 48	9 to 23	2 to 4	„
Esser	5	25	35	29	6	Children
Orland ...	7	28	39	16	0	„

The figures given by these observers agree on the whole fairly well, and can therefore be accepted as averages for normal individuals living in a temperate climate. According to the observations of Arneth, the numerical relationship of the neutrophile leucocytes becomes altered under the influence of certain infectious diseases in the sense that the numbers belonging to the first two classes increase as compared with normal conditions. This change he termed a 'shift to the left.'

The question has since been extensively studied, and most of the observers obtained analogous results.

Chamberlain and Vedder (1911), during their investigations on the effect of a tropical climate on white men, performed seventy-two Arneth counts on American soldiers, and for comparison fifty on Filipinos. Their work led them to the conclusion that 'the average Arneth picture showed a marked shift to the left in the case of Filipinos and a slight drift in the same direction for Americans resident more than one year in the Philippines.'

The differential and Arneth counts on two groups of American soldiers—one consisting of twenty-eight pronounced blondes and the other of twenty-eight pronounced brunettes—did not reveal any material difference between the two types.

When comparing Chamberlain and Vedder's results, obtained on Americans in the Philippines, with the normal figures given by European observers (comp. Table 3), it becomes evident that the differences are sufficiently pronounced to justify their conclusion.

TABLE 3

	Arneth classification per cent.					Index Arneth
	I	II	III	IV	V	
Normal in Europe ... ... (Arneth)	5	35	41.	17	2	40·0
Average for 72 healthy Americans in Philippine Islands	13·3	32·9	37·2	14·6	2	46·2
Average for 50 Filipinos	27·5	38·3	25·8	7·5	0·9	65·8

METHODS

The first few Arneth counts were performed by both of us on the same subjects, and were taken into consideration only after the results had become practically identical.

The blood films were stained by Giemsa's solution which, according to Pappenheim (1909), has certain advantages over Ehrlich's triacid. With the latter the nuclei stain indistinctly by the methyl green, and are more or less hidden by the darkly staining neutrophile granules; their contours are therefore difficult to distinguish. Giemsa's method has the additional advantage that the nuclei of leucocytes at the same stage of development are invariably stained to the same depth, and the connecting threads are sharply defined (Schilling-Torgau).

Two nuclear fragments, connected by a thread only, were counted separately, whilst they were counted as one when connected by a distinct bridge. In case of uncertainty regarding the class to which a.cell belonged, the higher class was chosen. Only perfectly prepared films were used, and in each case 200 successive cells were enumerated. Each 100 was counted separately, and when the figures were consistent the count was accepted.

The blood films were made about the same hour of the day, in order to make the results as nearly comparable as possible. For comparison a few Arneth counts were made on recent arrivals from a temperate climate, and these differed but little from the averages given by Arneth.

RESULTS OF ARNETH COUNTS ON 150 CHILDREN

Arneth and differential counts were performed on the blood of 150 children (boys and girls) of ages between 7 and 15, and tabulated according to the ages (comp. Table 4). For completeness the average numbers of erythrocytes, leucocytes and the average haemoglobin value (Fleischl-Miescher) for the same children are appended, counts which have been included amongst the average figures referred to in our communication on the general blood condition of children of European descent residing in tropical Australia. The children were not selected in any way.

The individual counts were fairly consistent and did not vary to any great extent. After the completion of the first twenty-five counts the averages were computed, and have been found to agree almost completely with the total average of the 150 Arneth counts.

The averages given in the table indicate that the Arneth index of normal healthy children, who have resided in tropical Queensland for the whole or most of their life is very high indeed, when compared with the corresponding figure of children living in a temperate climate; in other words, the Arneth blood picture of North Queensland children shows a well-marked 'shift to the left.'

An attempt was made to represent graphically our results and, for comparison, those of previous observers on normal Europeans (Arneth), on American soldiers in the Philippines, and for Filipinos (Chart 1). In this chart the classes are plotted out as abscissae and the percentages as ordinates; for the purpose of distinctness the points are connected by lines.

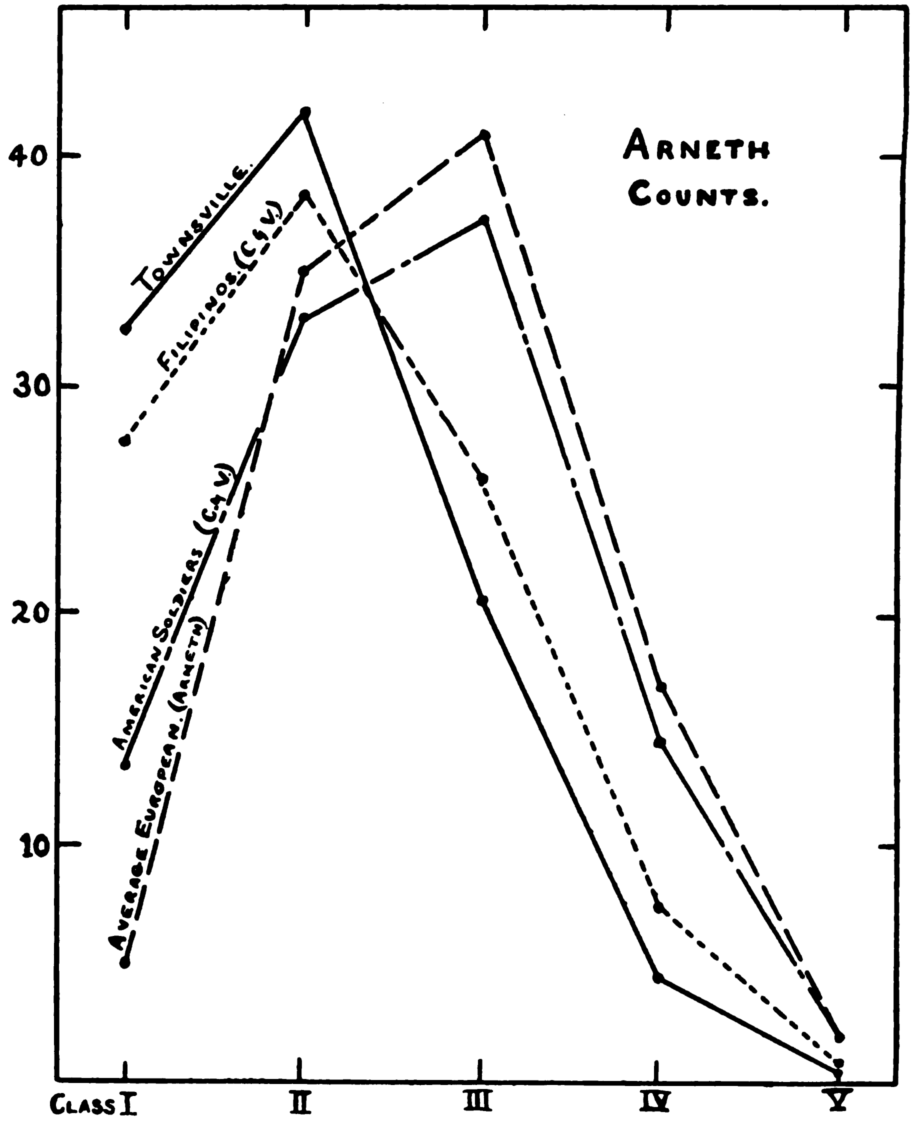


CHART I.

The graph brings out clearly that residence in the tropics influences the Arneth blood picture in a manner analogous to certain infectious diseases.

The 'shift to the left' after a short residence is only slight (as shown by Chamberlain and Vedder's figures for American soldiers in the Philippines); it becomes more pronounced after a prolonged residence (as shown by the figures for Townsville children). In fact, the Arneth index of North Queensland children resembles closely that of Filipinos.

We have thus, in the altered Arneth picture, the only definite indication of the influence of a tropical climate *per se* upon the blood conditions of Europeans.

Most authors consider that a 'shift to the left' in the Arneth blood picture is the expression of a lowered resistance of the body to infection.

They base this conclusion upon the observation that numerous Arneth counts performed on patients suffering from phthisis showed a close relationship between the course of the disease and the number of neutrophile leucocytes in the lower classes. This, however, does not justify the assumption that the leucocytes of the lower classes are less actively phagocytic than those in the higher classes. If it were so, phagocytic experiments *in vitro* should reveal a distinct difference in the phagocytic power of neutrophile leucocytes of the different classes.

Busse (1910) carried out researches on this point and found that 'the leucocytes obtained from healthy human blood, from inflammatory and post-operative leucocytosis, show exactly the same phagocytic power.' Our tentative experiments in this direction confirmed his observation, and bear out the statement that the conception of Arneth, namely, that the leucocytes with a lesser number of nuclear particles play a less important rôle for the protection of the body, does not hold good for phagocytic experiments *in vitro*.

In our opinion, the Arneth picture is an expression of the functional activity of the leucopoetic system, especially the bone-marrow, rather than that of phagocytic activity. The presence of a large number of neutrophile leucocytes belonging to class one indicates a greater activity of the bone-marrow, but does not necessarily imply that the resistance of the organism is lowered in any way.

Chamberlain and Vedder consider that 'in Filipinos this reduction in circulating phagocytic cells may be a visible indication of a lowered resistance to infection on the part of native races.'

In our experience, there is amongst the children in North Queensland (Townsville) no greater susceptibility to infectious diseases than amongst the same class in Europe.

That natives are more prone to contract infectious diseases, imported by Europeans, is not the expression of a lowered resistance, generally speaking, but is the expression of the lack of acquired active immunity, enjoyed by the population in places where the diseases in question had been existent for centuries. In the same way Europeans emigrating to the tropics are more prone to contract diseases peculiar to the tropics and in a more severe form than the natives.

The similarity of the Arneth blood picture in North Queensland children with that of Filipinos does not, in our opinion, mean a lowered resistance to infection, but is in all probability the outcome of purely climatic influences.

DIFFERENTIAL LEUCOCYTE COUNTS

For the differential count 500 successive leucocytes were enumerated, and the results included in Table 4.

The figures obtained for the different classes resemble those obtained for normal persons in Europe. The average number of the polymorphonuclear neutrophile leucocytes shows slight changes, being somewhat lower than usually accepted. It must, however, be kept in mind that there are discrepancies in the results published by different authors. Whereas Ehrlich and Lazarus (1910), and Arpad v. Torday, consider 65 to 70 per cent. as the normal average number of polymorphonuclear leucocytes, the averages of Hewes (1904) (55 to 65 per cent.) and of Bunting (1911) (54·6 per cent.) are somewhat lower.

When taking our individual differential counts into consideration, great variations are noticed in the children, the numbers of polymorphonuclear leucocytes ranging between 33 and 73 per cent., being in 26·6 per cent. of the children below 50 per cent. of the total number. On the whole there is, therefore, a distinct decrease in the number of this class of leucocytes.

TABLE 4.—Arneth and differential leucocyte counts on 150 children of North Queensland according to age.

Age	No.	Arneth classification per cent.					Arneth Index	Differential counts per cent.						Average Total No. of Leucocytes	Average Total No. of Erythrocytes	Average Haemoglobin	
		I.	II.	III.	IV.	V.		Polymorphonuclear	Transitionals	Large Mononuclear	Lymphocytes	Eosinophiles	Mast Cells			Absolute Value	per cent normal
7	6	35·5	43·3	17·6	3·5	0·3	78·8	55·9	5·6	1·4	30·8	6·2	0·1	10,816	4,971,200	12·09	87·5
8	12	35·2	41·6	18·8	4·1	0·3	76·8	52·2	6·3	2·7	28·9	9·9	0·00	9,794	5,169,300	11·63	84·5
9	18	30·6	41·3	23·2	4·4	0·5	71·9	58·6	4·5	2·5	28·0	6·3	0·02	12,244	4,696,500	12·15	88·0
10	20	32·7	42·0	20·0	5·0	0·3	74·7	56·1	4·3	2·2	30·1	7·3	0·0	10,173	4,987,400	12·21	89·0
11	17	30·1	45·3	19·6	4·6	0·4	75·4	55·5	3·3	1·8	31·7	7·6	0·05	9,627	4,955,300	12·12	88·0
12	30	33·8	41·7	20·1	4·0	0·4	75·5	57·4	4·7	3·2	26·3	8·5	0·05	10,370	5,132,400	12·55	91·0
13	33	31·0	41·3	22·5	4·7	0·5	72·3	54·2	3·2	2·2	32·3	8·0	0·03	10,544	5,073,700	12·61	91·5
14	11	34·9	42·4	18·9	3·3	0·5	77·3	59·6	3·2	2·5	27·0	7·6	0·03	10,351	5,256,700	12·95	94·0
15	3	33·3	41·0	19·3	5·7	0·7	74·3	56·8	5·1	1·1	31·9	4·9	0·1	10,555	5,421,900	12·53	91·0
Total Avge.	150	32·5	42·0	20·6	4·5	0·4	74·5	56·1	4·2	2·4	29·5	7·7	0·04	10,571	5,039,800	12·28	89%

This observation is in accordance with the finding of Chamberlain and Vedder, who obtained an average of 56·1 per cent. for the polymorphonuclear leucocytes of American soldiers in the Philippines in comparison with 56·8 per cent. for the Townsville children.

The average number of the eosinophile leucocytes is distinctly increased. Whereas 2 to 4 per cent. is considered normal in Europe, our average is 7·7 per cent., which is the more striking, since children suffering from helminthic infections had been as far as possible excluded.

The small number of brunettes amongst the children makes it impossible to draw any definite conclusions in respect to complexion and type.

CONCLUSIONS

1. The number of polymorphonuclear neutrophile leucocytes in the blood of children living in tropical Queensland is distinctly decreased, the number of the eosinophile leucocytes markedly increased.
2. The Arneth count performed on 150 school children living in tropical Queensland shows a marked shift to the left, the Arneth index being 74·5 as compared to 40 for normal individuals (Arneth) in Europe.
3. The 'shift to the left' in the Arneth blood picture is, in our opinion, due to the effect of a tropical climate upon the white race living in the tropics.

REFERENCES

ARNETH (1904). Die neutrophilen weissen Blutkörperchen. Jena.

ARPAD v. TORDAY (1913). Vom normalen Blutbild. Virchow's Archiv., Bd. 213, p. 529.

BUNTING (1911). The normal differential leucocyte count. Amer. Journ. of Med. Science, Nov.

BUSHNELL and TREUHOLZ (1908). Arneth method in the study of pulmonary Tuberculosis. Med. Rec., 73, p. 471.

BUSSE (1910). Phagozytose und Arneth's ches Blutbild. Münch. Med. Wschft., No. 2, p. 70.

CHAMBERLAIN and VEDDER (1911). A Study of Arneth's nuclear classification of the neutrophiles in healthy adult males and the influence thereon of race, complexion, and tropical residence. The Philippine Journ. of Science, Vol. VI, p. 403.

EHRLICH and LAZARUS (1910). Anaemia. London.

ESSER (1906). Das neutrophile Blutbild beim natürlich und Künstlich genährtem Säugling. Münch. med. Wochenschrift, No. 34.

HEWES, H. F. (1904). Standard records of the leucocytes in normal blood for reference in clinical work. Boston Med. and Surg. Journ., Dec., p. 705. (Rev. Folia Haematol., Vol. II, p. 325, 1905).

ORLAND (1907). Beiträge zur Untersuchung des neutrophilen Blutbildes beim gesunden und Kranken Säugling. Inauj. Dissert. Bonn, and Med. Klinik, No. 49.

PAPPENHEIM, A. (1909). Folia haematologica, Vol. VII, p. 85.

PAULICEK, E. (1907). Zur qualitativen Blutuntersuchung nach der von Arneth angegebenen Methode. Folia haemotologica, Vol. IV, p. 751.

SCHILLING-TORGAU (1911). Kritik der Arneth chen Lehre von der verschiebung des leucocytären Blutbildes und Wertung ihrer Klinischen Anwendbarkeit. Folia haematologica Archiv., Vol. XII, No. 1, p. 130.

SIMON, C. E. (1907). Clinical Diagnosis, 6th Ed., pp. 76 and 81.

THE OCCURRENCE OF LEAD POISONING AMONGST NORTH QUEENSLAND CHILDREN

BY

A. BREINL

AND

W. J. YOUNG

FROM THE AUSTRALIAN INSTITUTE OF TROPICAL MEDICINE

(*Received for publication* 28 *August*, 1914)

TWO CHARTS

INTRODUCTION

In 1892, Lockhart Gibson, Wilton Love, Jeffris Turner and others drew attention to a disease occurring amongst children living in the neighbourhood of Brisbane, which they attributed to lead poisoning.

The symptoms of the disease in the ten children mentioned was a paralysis of certain groups of muscles, namely, the extensors of the fingers, the tibialis anticus and the long extensors of the toes, and the muscles which form the bulk of the thenar prominence, the abductor opponens and flexor pollicis brevis.

The electrical examination in three cases revealed a well-marked reaction of degeneration in the paralysed muscles. Besides the paralysis, an anaemia of varying degree was observed; the characteristic blue line in the gum was only rarely seen, and when present occurred opposite a few isolated teeth only; pains in the limbs and spasmodic contractions of the calf muscles were most uncommon. None of the cases complained of colic, but four cases gave a clear history of gastrointestinal seizures and constipation. The diagnosis was based on the typical distribution of the palsy and the presence, in two cases, of distinct traces of lead in the urine after the commencement of the administration of potassium iodide. The question of the source of the poisoning was left in abeyance.

On the same occasion Jeffris Turner (1892) recorded four cases which lead him to the diagnosis of a localised meningitis. The children complained at first of headache and vomiting, and shortly afterwards an internal squint developed accompanied by a double optic neuritis, the outlines of the disc being totally obscured. The diagnosis of a slow-growing tubercle could be excluded, since pyrexia, convulsions and delirium were absent, and three of the four cases completely recovered whilst the fourth case improved slightly. The history and the course of the disease excluded syphilis, and localised basal meningitis of unknown origin was suspected.

Later, in 1897, Jeffris Turner divided the cases of lead poisoning in children into four groups, namely:

1. Paralytic cases showing symmetrical wrist and foot drop, spasms of the calf muscles, and as a secondary lesion a persistent talipes equinus.
2. Cases characterised by pains in the abdomen and limbs, concomitant with habitual constipation and an occasional blue line on the gums, the children becoming at the same time irritable and neurotic.
3. Eclamptic cases suffering from severe and persistent convulsions, which often end fatally.
4. Children suffering from ocular neuritis, a neuritis involving the optic and at the same time the oculomotor nerves.

Lead was found in the urine sometimes in minute traces, sometimes in appreciable quantities. According to Turner, seventy-six cases, fifty-five girls and twenty-one boys, were treated in the Brisbane Children's Hospital during six years, their ages varying between 6 and 12 years. As a number of the children who left the hospital apparently cured were re-admitted after an interval of a few months, the conclusion that the source of the poisoning was in the respective homes seemed beyond doubt. For drinking purposes, rain-water collected from the roofs in tanks had been used. The presence of lead in three different samples of tank water from houses where cases had occurred, examined after several months of dry weather, convinced the author that lead contained in the tank water was the source of the poisoning.

In the same year Lockhart Gibson (1897) published the histories of a few cases suffering from double optic neuritis, and simultaneously from paralysis of one external rectus. One of the children showed at the same time marked wrist drop and a tendency to ankle drop, another the blue line on the gums and traces of lead in the urine, and a less severe case distinct traces of lead in the urine, of which two specimens were examined. Lead poisoning was therefore thought to be the cause of the eye symptoms. The number of cases (twenty-four since 1891), the discovery of lead in the urine, the occurrence of colic, lead palsy, occurring simultaneously with the eye symptoms, the uniform absence of fever, the fact that no case which exhibited the symptoms attributed to ocular neuritis ended fatally, seemed to prove beyond doubt that lead was the cause of the disease in question, and tank water was considered as the source from which the lead was obtained. The fact that as a rule only one child of the household was attacked was attributed either to an increased sensitiveness of particular nerves to the lead, circulating in their capillary network, or to a deficient excretion of lead.

Three cases suffering from similar clinical symptoms, which ended fatally, were described by Green (1897). The pulse of these cases was rapid, the temperature above 100, rising to 105 before death. The post-mortem held on one showed an interstitial nephritis and a congestion of the cortex of the brain.

In 1899, Jeffris Turner amplified his observations. Lead, according to him, appeared to be only occasionally present in the urine, and then as a rule in extremely minute quantities. The characteristic sign of lead poisoning, the blue line on the gums, commenced as minute blackish dots opposite certain teeth only. He drew attention to the intense mental irritability and to the rapid wasting observed in severe cases, and to the common occurrence of pains in the limbs as well as constipation.

In 1904, Lockhart Gibson reported four cases of lead poisoning in children, with ocular neuritis. In two of them lead was found in the urine, 0·4 mg. and 0·32 mg. per litre urine respectively. Further observations convinced him that tank water could be practically excluded, and the lead paint of the walls and verandah railings was substituted as the source of infection.

In his conclusions he pointed out that the majority, probably all, of the children affected with lead poisoning had lived in houses in which the rooms, or at least the verandah railings, had been painted with lead paint.

Two conditions of painted surfaces would be more than usually liable to induce poisoning, viz.:

(*a*) Freshly painted or at least sticky surfaces.

(*b*) Painted surfaces which either by exposure to the sun and air, or for other reasons, had lost their oil and the paint had become a dry powder which was easily detached when rubbed, and intermingled with the dust of the room.

Dust, of course, is capable of being both swallowed and inhaled. The greatest danger, in his opinion, was the adhesion of the paint either by nature of its stickiness or of its powdery character to the fingers and nails, whence it is conveyed to the mouths of children, especially in those who bite their nails, suck their fingers, or eat with unwashed hands.

A further danger, in his opinion, worthy of consideration, was the absorption of the soluble lead salts contained in paint by the skin of the children's hands and feet.

He further quoted the case of two children who developed typical signs of lead poisoning shortly after having moved into a new house recently painted, and these two children were, according to the mother's statement, the only members of the family addicted to biting their nails.

An inquiry was therefore begun amongst the parents of undoubted lead cases—twelve in number—and their answers, ten in number, elicited the information that eight of the children were in the habit of biting their nails or sucking their fingers.

It was found by consulting the records of the Children's Hospital in Brisbane that from 1898 to 1903 eighty-five cases of lead poisoning had been treated as in-patients in the hospital, and of these forty-two were admitted during the months of December, January and February—the hottest months; twenty-eight were admitted during October, November, March and April; and sixteen only were admitted during the five cooler months.

A further contribution to this question was Dr. Jeffris Turner's Presidential Address on 'Lead Poisoning in Childhood,' which sums up the question up to the year 1909. Clinically he distinguishes cases with gastro-intestinal symptoms, with paralysis of the muscles of the limbs, with diaphragmatic and cardiac paralysis, with remote effects as anaemia, albuminuria, with eclampsia, acute optic neuritis associated with oculomotor paresis, and finally with chronic optic neuritis and atrophy without paresis of the sixth nerve.

Lockhart Gibson (1913) recommends the lumbar puncture, in all cases of lead poisoning where eye symptoms are prominent, as the best mode of treatment, since in his experience there was, in the majority of cases, a distinct increased tension of the cerebro-spinal fluid, and lumbar puncture seemed to relieve the paralytic symptoms considerably.

OBSERVATIONS IN TOWNSVILLE

Opportunity offered itself in Townsville to observe cases resembling those described by Jeffris Turner, Lockhart Gibson, and others in children admitted to the hospital.

Unfortunately during the first three years no chemical analysis of the urine for lead could be carried out. As the characteristic signs of lead poisoning, the blue line on the gums and drop-wrist, were only rarely met with, and as in the first cases basophilic granulations in the erythrocytes, which are generally considered as characteristic of lead poisoning, were absent from the blood, considerable doubt was felt about lead being the etiology of the cases in question. The chemical analyses of the cases previously reported in the literature seemed incomplete, and moreover, only in a few of the cases any reference at all was made to the presence of lead in the urine. Besides, no urine of normal children had been examined for the presence of lead, and in no case had any analysis of the faeces been made.

The clinical symptoms alone were barely sufficient to warrant the diagnosis of lead poisoning, especially as our knowledge of the clinical picture of lead poisoning in children is very incomplete.

Within the last year, however, the urine and faeces of a number of children clinically suspected as suffering from lead poisoning have been examined chemically, and the results have proved beyond doubt that lead poisoning is not of uncommon occurrence among children. During 1913 the excreta of twenty-two children were examined for the presence of lead, and in eighteen of these lead was found in varying quantities.

The symptoms observed corresponded, on the whole, to those previously described.

The cases may be classed according to the severity of the symptoms. The very early cases have no definite symptoms. Parents complain of a change in the character of the children within the last few weeks; whilst previously they had been normal and well-behaved, lately they had become peevish and fretful, were very restless at night, and had lost their appetite completely. As a rule only careful cross-questioning elicited the information that the child had been complaining of slight pains in the epigastrium and pains in the legs. The breath was often foul, and now and again ulceration was noticed in the mouth. According to the parents' statements, the children were flushed in their faces and appeared feverish. All the symptoms referred to are very indefinite and may be considered as the commencement of any infectious disease.

Even in these early cases lead can be found in the urine often in appreciable quantities, and without the chemical analysis the correct diagnosis would be very difficult.

In cases of slightly longer duration the pains in the abdomen, intermittent at first, have become more continuous. Constipation is the rule, but in rare cases a history of intermittent diarrhoea is given. Vomiting is only rarely observed. The pains in the calf of the legs become more marked, the calf muscles are often so painful that the little patients cannot endure even the weight of the bed-clothes, and at this stage the gait of the child becomes affected.

The Burtonian line was observed only in a small percentage of the children. This 'blue line,' due to the interaction of lead salts which have gained access to the mouth, and the sulphuretted hydrogen produced by the decomposition and putrefaction of food, is at present regarded 'of no more value than that the person showing such pigmented gums has been exposed to lead absorption.' (Legge and Goadby, 1912.)

A continuous line is not often found in children, but the characteristic minute black spots occur around teeth which are coated with a deposit of tartar, requiring the use of a hand-lens for their diagnosis.

Many of the cases showed foot- and wrist-drop. As pointed out by other observers, certain nerves of the legs are as a rule affected first, thus differing from the clinical symptoms of lead poisoning in adults, where drop-foot is but rarely present.

The extensors of the toes and the peronei muscles are most frequently involved. The patients have a characteristic gait; they walk on the outside of their feet, the gait is high-stepping, the toes are dragged along the ground, and at each step the legs are swung sideways before again being put down to the ground.

Drop-wrist due to a paralysis of the extensor communis digitorum is not rare. In two advanced cases the Aran-Duchenne type of paralysis was present, in which the muscles of the thenar and hypothenar eminences and the interossei showed marked atrophy. In the majority of cases with nervous symptoms a slight atrophy of the muscles in question was noticeable.

The eye symptoms described by Lockhart Gibson were only present in one out of the twenty-two cases examined. This child complained of dimness of vision, but an examination of the fundus was impossible on account of the restlessness of the child.

Brain symptoms are common, ranging from obstreperousness and fretfulness to a state resembling mania. As already remarked, the parents invariably complain of a marked change in the character of the previously well-behaved children. In two extreme cases the children had become quite unmanageable since their illness. They screamed day and night, and kicked at everybody who came near them. Shortly after their removal from home, and after a vigorous treatment with potassium iodide, these children again became quite normal.

Not rarely convulsions are the outcome of chronic lead poisoning in children, and these do not differ in any way from convulsions due to other causes, except in their severity. In one case the convulsions, after admission to the hospital, continued for three-quarters of an hour, and only abated after the administration of large doses of chloral hydrate and bromides.

Albumen was not found in the urine of any of the cases.

The body temperature is often normal throughout, sometimes irregular, but very rarely rising above 101° F. (see Chart 1). The pulse rate is invariably high in spite of normal temperature (comp. Chart 2). Respiration is normal.

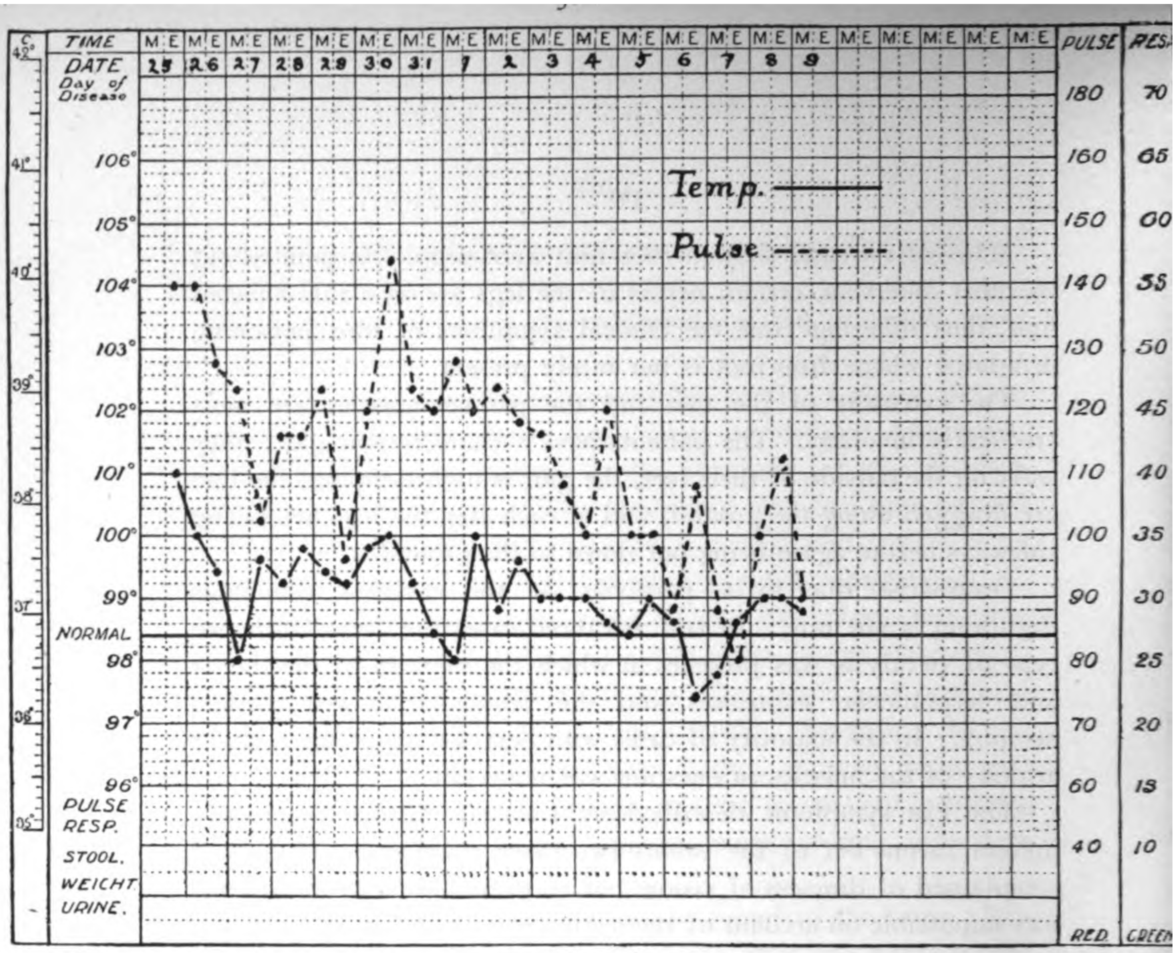


CHART I.

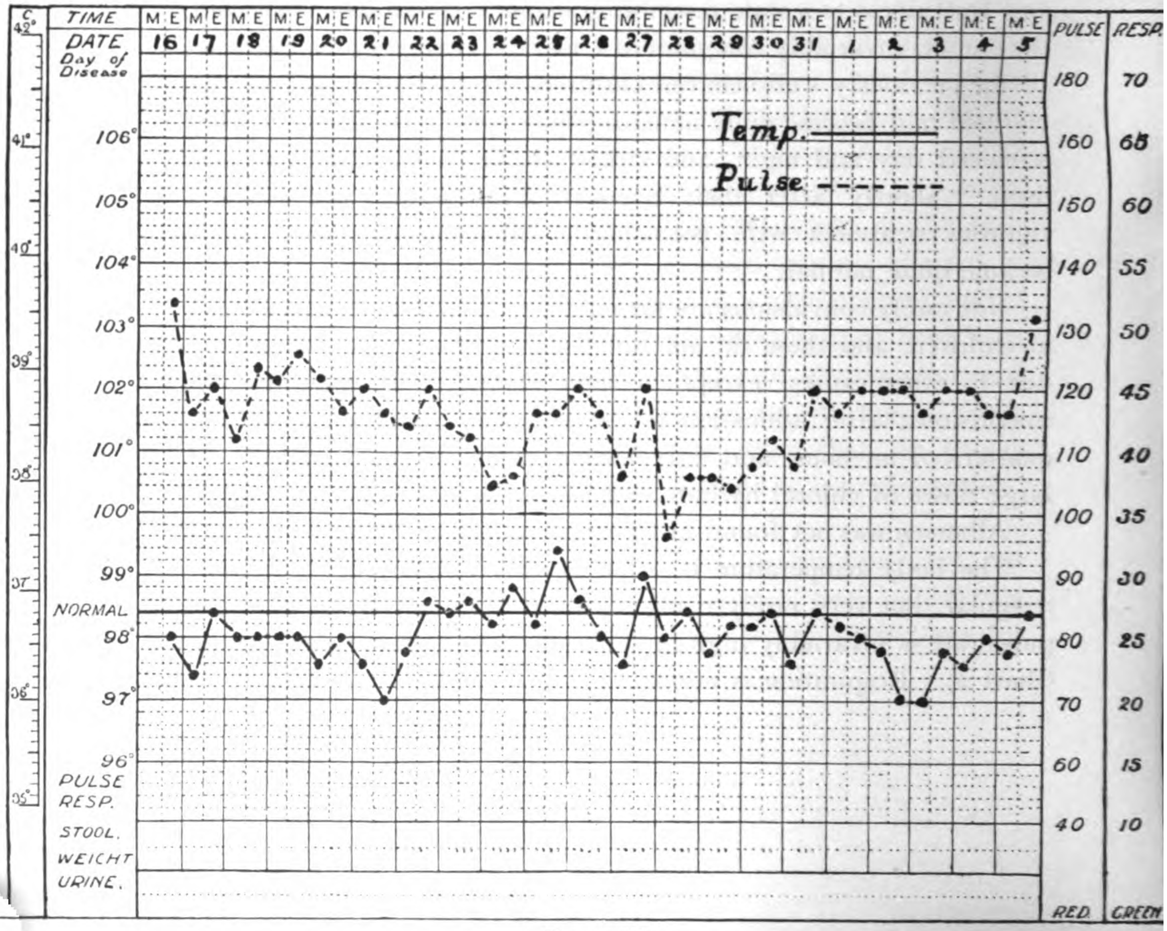


CHART II.

Anaemia is only marked in cases of long standing. Blood counts in early cases reveal normal conditions; in chronic cases the number of red cells may be diminished; the lowest count gave 3,000,000 red blood corpuscles with a haemoglobin of 9·34 grams in 100 c.cm. blood.

Basophilic granulations in a small number of erythrocytes were found in the majority of cases, sometimes only after a prolonged search.

COMPARISON OF THE SYMPTOMS OF LEAD POISONING IN CHILDREN AND ADULTS

The clinical symptoms of lead poisoning in children differ in many ways from plumbism in adults.

The occurrence of the blue line and colic is common to all ages. Whereas in adults the palsy affects the upper limbs most frequently, and the lower limbs only in a proportion of 13 to 100 (Tanquerel, 1913), in children the first symptoms of paralysis appear in the lower limbs, the arms becoming affected later. Cerebral symptoms resembling a mild form of mania are not at all uncommon in any age.

The pulse in lead poisoning in adults, according to Legge and Goadby (1912), may be increased during the early stages only. In children a quick and small pulse is the rule.

The occurrence of eye symptoms such as optic neuritis is an exception in adults (Legge and Goadby), whereas in children eye symptoms in the form of oculomotor neuritis are common (Lockhart Gibson).

The mucous membrane of the mouth and gums in children is often inflamed, and shows inclination to formation of sores.

In the same way as adults, children are prone to relapses in cases where they have returned to the locality where they first became affected, and many such children are readmitted to the hospital at irregular intervals.

PROGNOSIS

The prognosis of lead poisoning in children in the early stages is, on the whole, good. The colic becomes less violent and the pains in the legs disappear completely. As in adults, the complaint becomes more serious with the second and third attack. Whereas children treated in the early stages recover the use of their limbs completely in the course of a few weeks, in the latter stages it requires months before they are able to walk. In many instances the patients remain crippled for life, in spite of careful treatment.

The slight mental disturbances so marked in the early cases abate with the general improvement, and even the severe cerebral symptoms, such as convulsions and mania, disappear after treatment for a comparatively short time.

CHEMICAL EXAMINATION OF THE EXCRETA

The urine and faeces of cases clinically suspected of lead poisoning were examined chemically. Although lead is often present in the urine of cases which have been exposed to lead absorption, especially after the administration of potassium iodide, the results of numerous investigations have shown that this is not invariably the case, and that even when lead is present it only occurs in small amounts.

Thus Legge and Goadby (1912) state that 'the presence of lead in the urine is not so common nor so definite a symptom as might be supposed,' whilst Jaksch (1901) goes so far as to assert that no lead at all is excreted in the urine of patients suffering from chronic lead poisoning, but only in the acute stages. Dixon Mann (1908) described a similar case in which during treatment with potassium iodide the excreta were analysed separately, with the result that much larger quantities of lead were found in the faeces than in the urine. When lead salts were administered to the same patient, the bulk of the lead appeared in the faeces. These observations led him to the conclusion that lead salts are mainly excreted into the intestine and eliminated with the faeces. Experiments carried out by Legge and Goadby and other investigators confirmed this view.

It appeared therefore desirable to examine the faeces as well as the urine, and the results obtained justified this proceeding. In every case a larger quantity of lead was found in the faeces, and lead was often present in these when no trace could be detected in the urine.

For the chemical analysis the material was treated in the following manner: The organic matter in the urine and faeces was oxidised by the well-known method of Fresenius and Babo with hydrochloric acid and potassium chlorate. The resulting liquid was evaporated to dryness, and the main excess of hydrochloric acid removed by repeatedly adding distilled water and evaporating down. This was then almost neutralised with sodium bicarbonate, filtered hot, and the faintly acid solution saturated with sulphuretted hydrogen. Any lead sulphide was filtered off, washed with sulphuretted hydrogen water, and dissolved by repeatedly evaporating to dryness; the residue was taken up in water, and the lead estimated colorimetrically. The brown colour given on addition of sulphuretted hydrogen water in the presence of potassium hydrate was compared with that given under similar conditions by a standard solution of lead nitrate. When the quantity of lead present exceeded one milligramme, it was re-dissolved in nitric acid and estimated gravimetrically as lead sulphate.

In every case confirmatory qualitative tests for lead were made on one sample of the solution in nitric acid obtained as above. The tests employed were the yellow precipitate with potassium chromate, white precipitate with sulphuric acid insoluble in cold nitric acid and soluble in ammoniacal ammonium tartrate, and the formation of microscopical violet-black cubical crystals of copper lead nitrate when the solution was mixed with copper acetate and potassium nitrate and allowed to evaporate.

The residue of fat and unoxidised matter which was filtered off after the oxidation with hydrochloric acid and potassium chlorate would contain any lead sulphate which might be present in the faeces or urine. It was therefore treated with hot ammoniacal ammonium tartrate, and the filtered liquid evaporated to a small bulk, acidified with hydrochloric acid, and saturated with sulphuretted hydrogen. In no case did this residue contain more than a trace of lead.

The chemicals used in the analysis were carefully tested for lead, and only those free from lead were employed. Blank experiments were also carried out with normal children's urine in the vessels actually employed in the operations, and these yielded negative results throughout.

The quantities of lead obtained are given in Tables 1-3, the cases being classed according to the severity of the symptoms. In some instances the total excretion of urine and faeces during several definite periods were analysed separately. In others, however, urine was collected until a quantity sufficient for analysis (at least 1000 c.c.) had been obtained, and since the quantities of lead found do not represent the excretion over any definite period, the amount of lead per 1000 c.c. of urine is given.

TABLE 1.—Early cases in which the only symptoms were pains in the legs and epigastrium.

Particulars of Case.	Date.	Material Examined.	Lead Found.
1. G.M.—Girl, 12 years old... Potassium iodide commenced 29.2.13	25.6.13	1 day's urine	0·23 mgm.
	28.6.13	3 days' urine	0·70 ,,
	1.7.13	3 days' urine and faeces	3·2 ,,
	Dec., 1913	Urine	None.
2. R.D.—Boy, 13 years ... Before potassium iodide	29.7.13	2 days' faeces	6·8 mgm.
	30.7.13	1 day's urine	0·6 ,,
	1.8.13	1 day's faeces	0·2 ,,
	14.8.13	1 day's urine	None.
		1 day's faeces	Trace.
3. B.Y.—Girl, 4 years ... No potassium iodide ...	2.7.13	Urine.	0·32 mgm. per litre.
		1 day's urine.	0·43 ,,
4. J.H.—Boy, 10 years ...	21.7.13	Urine.	0·18 mgm. per litre.
		Faeces.	0·32 mgm.
5. H.H.—Girl, 9 years ...	16.8.13	Two stools.	1·7 mgm.
6. D.S.—Girl, 9 years ...	18.10.13	Urine.	0·32 mgm. per litre.
7. H.H.—Boy, 9 years ...	5.11.13	Urine.	0·24 mgm. per litre.
	6.11.13	Two stools.	2·4 mgm.
8. E.W.—Boy, 6 years ...	29.10.13	Urine.	0·69 mgm. per litre.

Table 2.—Cases with paralytic symptoms. (Drop wrist, drop foot.)

Particulars of Case	Date	Material Examined	Lead Found
9. D.O'B.—Girl, 4 years ... Receiving potassium iodide.	15.2.13	Urine.	0·37 mgm. per litre.
	28.2.13	Urine.	0·65 „
	2.3.13	2 days' urine	0·40 mgm.
	4.3.13	2 days' urine.	0·30 „
	6.3.13	2 days' urine.	0·35 „
10. E.B.—Girl, 6 years ... Treatment with potassium iodide commenced on 28.2.13 (Compare Chart 2.)	27.2.13	1 day's urine.	0·48 mgm.
		1 day's faeces.	1·27 „
	1.3.13	2 days' urine and faeces	3·2 „
	2.3.13	1 day's urine	None
		1 day's faeces	0·50 mgm.
	3.3.13	1 day's urine and faeces	1·3 „
	4.3.13	1 day's urine and faeces	0·50 „
	5.3.13	1 day's urine and faeces	0·42 „
	6.3.13	1 day's urine and faeces	1·9 „
	8.3.13	2 days' urine and faeces	0·22 „
	10.3.13	2 days' urine and faeces	1·7 „
11. M.R.—Girl, 3 years ... No potassium iodide	20.1.14	Urine	0·34 mgm. per litre.
	21.1.14	Two stools	1·2

Table 3.—Cases showing pronounced cerebral symptoms.

Particulars of Case	Date	Material Examined	Lead Found
12. E.McC.—Girl, 3 years ... Receiving potassium iodide	7.6.13	1 day's urine and faeces	1·9 mgm.
	10.6.13	3 days' urine and faeces	4·6 „
	12.6.13	2 days' urine and faeces	1·2 „
13. T.E.—Boy, 14 years ... ... No potassium iodide	9.1.14	Urine	0·49 mgm. per litre
14. B.E.—Boy, 6 years ... ... No potassium iodide	28.12.13	Urine	Trace only in litre
	30.12.13	Urine	0·45 mgm. per litre
15. M.—Girl, 6 years ... ... No potassium iodide ...	23.8.13	One stool	0·54 mgms.
	25.8.13	1 day's urine and faeces	0·30 „
16. L.H.—Boy, 4 years ... ... No potassium iodide	27.12.13	Sample of faeces and urine mixed	1·1 mgm.

The results of the chemical analysis show that the quantity of lead excreted is not always in direct proportion to the clinical symptoms of the case. It is thus not the amount of lead excreted, but the amount of lead retained in the body, which regulates the severity of the symptoms.

SUSCEPTIBILITY

Just as with adults, the individual susceptibility of children to lead poisoning shows great variation. It often happens that only one child out of a family of several develops symptoms of lead poisoning, although all have been exposed to the same degree of resorption.

Previous observers in Queensland have noted that where one child in a family is suffering from pronounced lead poisoning one or two of the others occasionally show only the blue line on the gum.

From this point of view in two instances chemical examination was made of urine of the brothers or sisters of the patient. In one case the urines of a baby and of the brother (approximately of the same age as the patient) living in the same house were analysed; the former was quite free from, and the latter showed traces of, lead. In the other case the urine of the brother was examined on two different occasions, and 0·6 mg. and a trace of lead respectively were detected.

In the light of these results the question arises as to whether lead in small quantities may be found normally in the urine of children living in houses coated with lead paint, clinical symptoms appearing only in those particularly susceptible to the poison. The complete failure to detect lead when the urine of a number of healthy children living under similar conditions was analysed, disproves this conception.

SOURCE OF THE LEAD

As to the source of the poison, Lockhart Gibson, quoted above, considered ingestion of paint the most likely one, although he recognised the possibility of dust containing lead entering the body by inhalation.

Experiments bearing on this question were carried out by Goadby and Goodbody (1909) and by Legge and Goadby (1912) which made it evident that the danger of lead inhaled as dust is many times greater than that of lead swallowed, and that the ratio of poisoning viâ the lungs to poisoning viâ the intestinal canal is 100: 1.

A limited number of experiments in the same direction have been carried out on monkeys in this laboratory. Two monkeys were fed with white lead paint as used for house decoration. Varying small quantities were administered every few days in the form of pills, or were smeared over the tongue or mucous membrane of the cheeks for about four months. Neither of the animals was the worse for the treatment.

These experiments suggest the probability that the inhalation of dust containing lead may be the more common source of poisoning in children, although observations here coincide with those of Lockhart Gibson, namely, that a great number of the children affected were addicted to biting their finger-nails.

Further experiments are, however, required before a definite pronouncement as to the way by which the lead enters the body can be made.

It is striking that cases similar to those described above should not have been recorded from other parts of the tropics where lead paint is employed. Whether this be due to local conditions prevalent in Queensland only, to the difficulty of diagnosis, or to the lack of a definite clue associating such symptoms with lead, remains to be seen.

REFERENCES

Gibson, Love, Hardie, P. Bancroft, Jeffris Turner (1892). Notes on lead poisoning as observed in children in Brisbane. Intercolonial Medical Congress of Australasia, Sydney. p. 76.

Gibson, Lockhart (1897). Ocular Neuritis simulating basal meningitis Plumbism. Australian Medical Gazette, Oct. 20th, p. 479.

——— (1904). A plea for painted railings and painted walls of rooms as the source of lead poisoning amongst Queensland children. Australian Medical Gazette, Vol. XXIII, p. 149.

——— (1913). The Importance of Lumbar Puncture in the plumbar ocular neuritis of children. Transactions of the Australasian Medical Congress, 1911, Vol. II, p. 750.

Goadby and Goodbody (1909). Seventh International Congress of Applied Chemistry, Section VIIIa, p. 306.

Green, J. E. (1897). Some unusual forms of lead poisoning. Australian Medical Gazette, Oct. 20th, p. 483.

Jaksch, R. v. (1901). Klinische Diagnostik, 5th ed.

Legge and Goadby (1912). Poisoning and lead absorption, London, p. 122.

Mann, Dixon (1908). Forensic Medicine and Toxicology. London, C. Griffin & Co. pp. 487-497.

Tanquerel (1913). Quoted by Osler, Principles and Practice of Medicine. London.

Turner, Jeffris (1892). A form of Cerebral disease characterised by definite symptoms, probably a localised basic Meningitis. Intercolonial Medical Congress of Australasia, Sydney, p. 98.

——— (1897). Lead poisoning among Queensland children. Australian Medical Gazette, Oct. 20th, p. 475.

——— (1899). How to recognise lead poisoning in children. Australian Medical Gazette, Vol. XVIII, p. 425.

——— (1909). Lead Poisoning in Childhood. Transactions of the 8th Session of the Australasian Medical Congress, Vol. III, No. 3, p. 1. Reprinted British Medical Journal, April 10th.

OBSERVATIONS ON THE BLOOD CONDITIONS OF CHILDREN OF EUROPEAN DESCENT RESIDING IN TROPICAL AUSTRALIA

BY
A. BREINL
AND
H. PRIESTLEY
FROM THE AUSTRALIAN INSTITUTE OF TROPICAL MEDICINE, TOWNSVILLE

(*Received for publication* 28 *August*, 1914)

THREE CHARTS

Tropical Anaemia, that is, an anaemia due to climatic influences after a prolonged residence in the tropics, was formerly an important factor in tropical pathology and was, up to comparatively recent times, a generally accepted condition. The striking pallor of the skin, so frequently encountered amongst Europeans residing in a humid tropical climate, was considered to be due to a deterioration of the blood.

It must, however, be kept in mind that this so-called tropical anaemia, inferred from the pale skin of tropical residents whose health had not suffered to any extent, was in many cases not differentiated from a secondary anaemia brought about by parasitic invasion, since the deleterious effect of a number of parasites upon the blood was not fully appreciated.

Marestang (1889, 1890) and van der Scheer (1890) undertook systematic blood examinations on a number of Europeans residing in the Dutch East Indies. They found, so far as conclusions could be drawn from their comparatively small number of blood counts, that the blood conditions of apparently healthy individuals in the tropics did not differ to any marked extent from those of Europeans living in a temperate zone.

Eijkman (1891) in the same way examined the blood of fifty-three Europeans who had resided in the Dutch East Indies for periods varying from two days to fourteen years, without finding any marked diminution in the number of formed elements nor in the amount of haemoglobin. The number of red blood corpuscles varied between 5,182,000 and 5,358,000, and the haemoglobin value between 96·5 and 100 per cent.; the average figures for the specific gravity were found to be 1·0574 and the water contents 77·93 per cent.

Glogner (1892) conducted investigations on similar lines amongst Europeans in Sumatra.

The average figure obtained for the red blood corpuscles of the fifty-one Europeans was 5,282,666, whilst for thirty natives the average was 5,578,000. The haemoglobin content of the blood of eighty Europeans, estimated by Fleischl's method, varied between 87·4 and 95·5 per cent., according to the distance of the light from the mirror and to the source of light (candle-light or kerosene lamp). The hemoglobin value for thirty-five natives varied between 90·2 and 91·9 per cent. The specific gravity of the blood of apparently normal Europeans was 1·0544 and of natives 1·055. Glogner concludes, in spite of these figures, that the number of red blood corpuscles and haemoglobin content of the blood of Europeans living in the tropics showed a slight diminution when compared with Europeans living in a temperate climate.

Plehn's (1892) observations are in accordance with Eijkman's results, showing that the blood of healthy Europeans in the tropics does not undergo any marked change.

Recently, similar investigations were undertaken on Americans in the Philippine Islands. Wickline (1908) computed the results of blood examinations of American soldiers in the Philippines. The first estimations were made after a residence of three months in the islands. There was a progressive fall in the percentage of haemoglobin and at the same time a rise in the number of red blood corpuscles, except at the last observation period, causing a decrease in the colour index (see Table 1).

Chamberlain (1911) collected 1,418 red cell counts and 1,433 haemoglobin estimations, performed on 702 soldiers who had been living for about twenty months in the Philippines. He concludes that 'the red cell counts (averaging 5,200,000, rarely falling below 4,500,000) do not differ from the normal at present recognised for healthy young men in the temperate zone,' and furthermore, that 'the haemoglobin percentage obtained by means of Dare's haemoglobinometer (averaging 89·6 per cent., rarely falling below 85 per cent.) and the colour index (averaging 0·86 or 0·87) are probably a little low, but not sufficiently so to indicate a definite anaemia.'

TABLE 1.—Wickline's average estimations of the erythrocytes and haemoglobin of American officers and soldiers who arrived in the Philippines in June, 1905.*

Number of men examined	Dates of examination	Average number of erythrocytes per cubic millimetre	Average haemoglobin per cent.	Average colour index
70	September, 1905	4,980,555	94	0·94
104	December, 1905	5,343,595	89	0·83
97	August, 1906	5,429,960	86	0·79
81	April, 1907	5,330,888	83	0·78

* Quoted from Chamberlain, Philippine Journal of Science, Vol. VI, No. 6, p. 483.

Cuthbert (1911) examined the blood of twenty-one European soldiers, who had not been ill during their years of residence on the west coast of Africa, without finding any diminution in the number of erythrocytes (average 5,550,190), a slight depreciation in the colour index (0·91), but too small to account for the pallid condition of the men.

These observations on the blood conditions of Europeans residing in the tropics are extremely interesting, but not entirely convincing, since the conclusions, with one exception, are based on a comparatively small number of blood counts and haemoglobin estimations, and the probable statistical error is therefore great. Chamberlain's averages were obtained from a sufficiently large number of blood counts (namely, 1,418), but his subjects were especially chosen young healthy American soldiers who had resided in the Philippines for a comparatively short time only—two years at the longest.

Most of the averages, given by the different observers quoted, are subject to the same criticism, as the majority of the persons had resided in the tropics for a limited period only.

Opportunity was therefore taken to make observations on similar lines in Townsville. In tropical Queensland a second and, to a limited extent, a third generation of children is growing up at the present time. A sufficiently large number of blood counts and haemoglobin estimations on children of ages between 7 and 15 years who had been residing in Townsville during the whole or most of their life would, in our opinion, furnish more convincing evidence on the influence of a tropical climate, as prevailing in the coastal belt of North Queensland, upon the blood conditions of a transplanted European race. Sufficient time would have elapsed for any alteration due to climatic influence only to become established.

TECHNIQUE

The technique of counting the formed elements of the blood at present in use is far from perfect, and the personal error may be considerable.

In order to eliminate, as far as possible, this personal equation, all the blood counts were performed by the two of us, and only after a number of preliminary counts on the same individuals had shown that the results of both of us were constant within a 5 per cent. limit.

For the counting of the red and white blood corpuscles the Thoma-Zeiss haemocytometer was used. For the erythrocytes the blood was diluted with Hayem's fluid in a proportion of 1 to 200, and the corpuscles in ten large squares, each consisting of twenty-five small squares, were enumerated.

For the total leucocyte count the blood was diluted in the proportion of one in twenty with a solution of half per cent. acetic acid in distilled water with the addition of a sufficient amount of a one per cent. solution of gentian violet, and the contents of 0·6 c.mm. counted. Blood from the ball of the finger was obtained by means of a broad lancet-shaped needle.

Our preliminary experiments proved that the manipulation for obtaining the blood was of great importance, since the results varied considerably with the amount of pressure, and only blood flowing freely was used for the estimations.

The haemoglobin was estimated by means of the Fleischl-Miescher haemoglobinometer, as supplied by Reichert of Vienna.

Two instruments were employed, one obtained at the end of 1909, the second two years later. The readings of the two instruments differed to such an extent that it was necessary to standardise the haemoglobinometers anew and discard the tables furnished by the maker.

The method used consisted of standardising a Haldane's haemoglobinometer by comparing the readings of this instrument with the results obtained by the determination of the oxygen capacity of several samples of blood by Haldane's ferricyanide method of the same blood on different occasions.

The haemoglobin contents of the same sample of blood was estimated by this standardised Haldane's apparatus and compared with the readings of the two Fleischl-Miescher instruments. Haldane's haemoglobinometer was used so as to have a permanent standard to determine whether the differences in the readings of our two Fleischl-Miescher instruments were due to fading of the ruby glass or to faulty graduation by the manufacturer.

The Fleischl-Miescher haemoglobinometer was preferred, as each estimation is calculated after two comparative readings, using two columns of haemoglobin dilution of proportionate depth.

High readings, over 85 per cent., are difficult, and would in some instances be inaccurate without the control of the smaller vessel.

Our experience of the different readings obtained with the two haemoglobinometers indicates the necessity for standardising every instrument in spite of the maker's assurance that each apparatus had been compared with a standard and the values calculated accordingly.

Haldane's haemoglobinometer without doubt gives the most accurate readings; unfortunately its use is restricted by the necessity of a coal gas supply for the preparation of the solution.

Gower's instrument was found to be useless in the tropics, since the carmine gelatine standard very soon becomes cloudy and loses its colour.

RESULTS OF THE BLOOD EXAMINATIONS

The results referred to in the following pages are based on 580 blood examinations performed on the same number of children of both sexes. No selection was made beyond rejecting the blood counts of those children who were found to be infected with Ancylostoma.

The children examined were of ages between 7 and 16 years. The number of children of the various ages differs; the small number of young children is explained by the difficulties of dealing with children so young.

Boys.

In Table 2 are found the average figures for erythrocytes, leucocytes and haemoglobin value for 305 boys. The average figures are practically constant.

The number of erythrocytes of the boys seven years of age is slightly higher than the corresponding one for boys one year older. This slight difference may be explained by the smaller number of boys examined, namely, ten in comparison with twenty-eight of the higher age.

The results of the individual counts are graphically represented in Chart 1; the upright column represents the percentage of children classified according to the number of their red blood corpuscles, each column signifying an increase of 250,000 corpuscles per c.mm. over the preceding column. The graph shows that out of the whole number of boys examined, 3·5 per cent. had an erythrocyte count lower than four millions, 42·4 per cent. between four and five millions, 48·7 per cent. between five and six millions, 4·8 per cent. above six millions; altogether 65·7 per cent. of the blood counts varied between 4·5 and 5·5 millions, which is generally considered the normal range.

The average figure for the red cells, namely, 5,046,100, does not represent the average of averages but was obtained by calculating the mean for the 305 individual counts.

The consideration of the length of residence of the children in tropical Queensland revealed the striking fact that the great majority of the children were born and bred north of the Capricorn and had not resided for any prolonged period in a temperate climate. Only nineteen boys of the 305 had been for less than three years in North Queensland; the average number of their erythrocytes was 4,984,100, which does not differ markedly from the mean figure, namely, 5,046,100.

TABLE 2.—Average figures of blood counts and haemoglobin estimations of 305 schoolboys, according to ages.

Age	Average number of erythrocytes	Number of estimations	Average number of leucocytes	Number of estimations	HAEMOGLOBIN			Number of estimations
					Average absolute content	Average percentage	Average colour index	
7 years ...	5,095,200	10	10,226	10	12·14	88%	0·87	9
8 ,, ...	4,981,400	28	10,286	28	11·91	87	0·87	28
9 ,, ...	4,905,400	38	12,063	38	12·09	87	0·88	38
10 ,, ...	5,020,200	48	11,095	45	12·18	88	0·87	47
11 ,, ...	5,025,900	37	10,790	35	11·92	87	0·86	38
12 ,, ...	5,094,100	49	11,078	46	12·04	87	0·86	51
13 ,, ...	5,176,800	51	10,272	50	12·76	92	0·89	54
14 ,, ...	5,141,700	38	10,521	38	12·68	92	0·89	40
15–16 ,, ...	5,032,200	6	9,594	6	12·94	94	0·93	6
Total average...	5,046,100	305	10,868	296	12·31	89%	0·88	311

The inquiry extended over a period of twelve months. It was pointed out by Taylor and Young (1914) that the climate of Townsville may be divided into two distinct seasons, a wet and dry season, the latter from May to October with a mean minimum

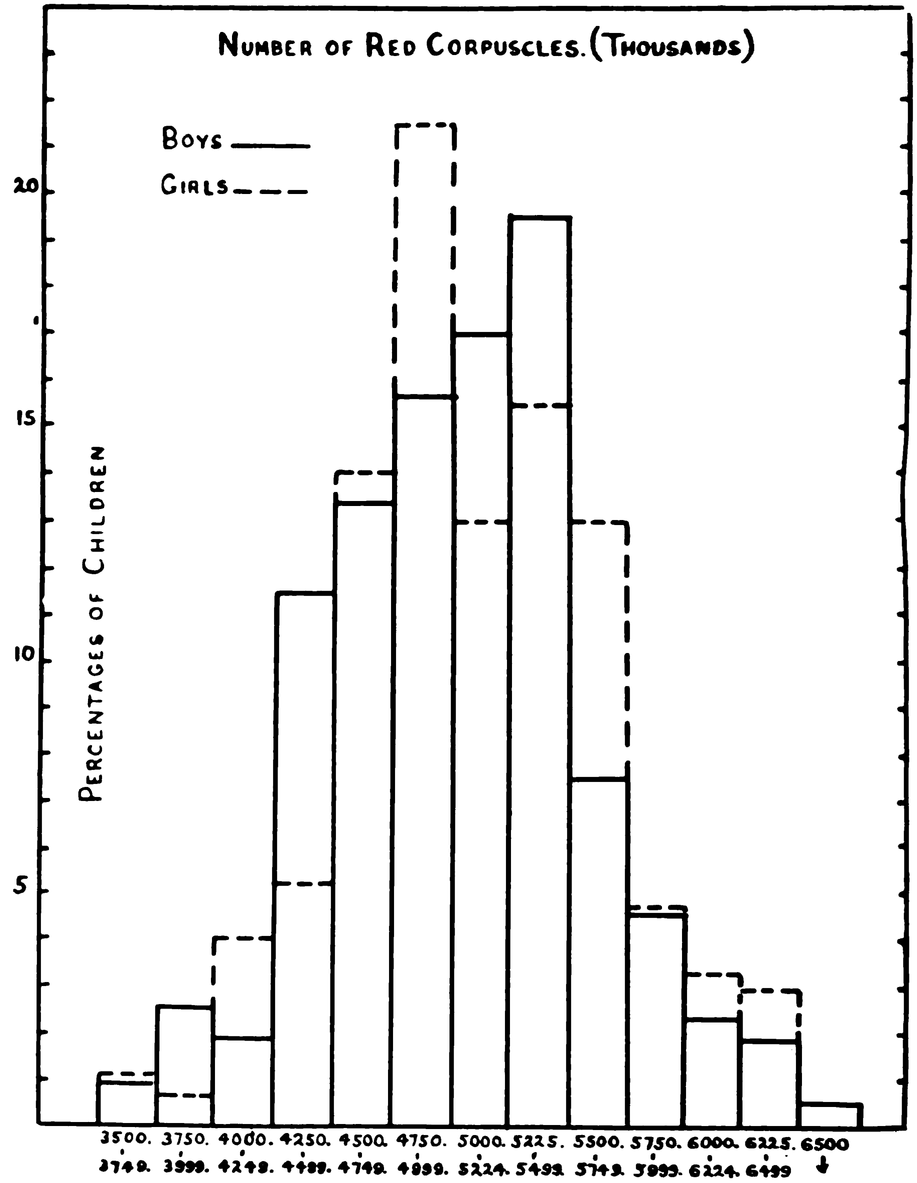


Chart I.

temperature of 61·2° F. and a mean maximum of 77·8° F., and a relative humidity of 62 per cent. The former, the wet season, extends from November to April, and has a mean minimum temperature of 74·7° F., a mean maximum of 86° F., and a mean relative humidity of 72 per cent. The wet season corresponds to an average tropical coastal climate, e.g., that of Ceylon, the dry season, on the other hand, to an average sub-tropical climate.

A comparison of the figures from the point of view of these seasonal variations proved that the climatic condition as such had no pronounced influence upon the number of red blood corpuscles present in the peripheral circulation. (Compare Table 3.)

TABLE 3.—Comparison of the average erythrocyte count and haemoglobin value of 305 schoolboys (311 resp.) during the wet and dry season.

Season	Average erythrocyte count	Number of boys	Haemoglobin			Number of estimations
			Average absolute content	Average percentage	Average colour index	
Wet season ...	4,959,600	129	12·34	89%	0·88	134
Dry season ...	5,096,900	176	12·28	89	0·87	177

A comparison of the averages for boys of dark and fair complexion indicates the absence of any differences in this respect, the figure for fair boys being 5,039,900 (203 blood counts), and for dark boys 5,061,800 (42 blood counts).

LEUCOCYTES

Leucocyte counts were performed on the same boys; the time chosen was between ten and twelve in the morning in order to obtain comparable results. Chart 2 shows that the number of white blood corpuscles of the individual counts showed a wide range; the averages for the different ages (compare Table 2) are, however, fairly constant.

Arneth (1904) asserts as a result of his extensive experience over a comparatively long period that the number of leucocytes (excluding the digestive leucocytosis) varied between 5,000 and 6,000, only rarely exceeding 6,000. If these figures are accepted, the school children in North Queensland have a marked leucocytosis. Other observers, however, amongst them Ehrlich and Lazarus (1910), regard any leucocyte count between 6,000 and 10,000 as normal. In any case the conclusion is justified that a great number of school-boys in North Queensland have a leucocytosis, 42 per cent. of 296 leucocyte counts ranging higher than 10,000 per c.mm.

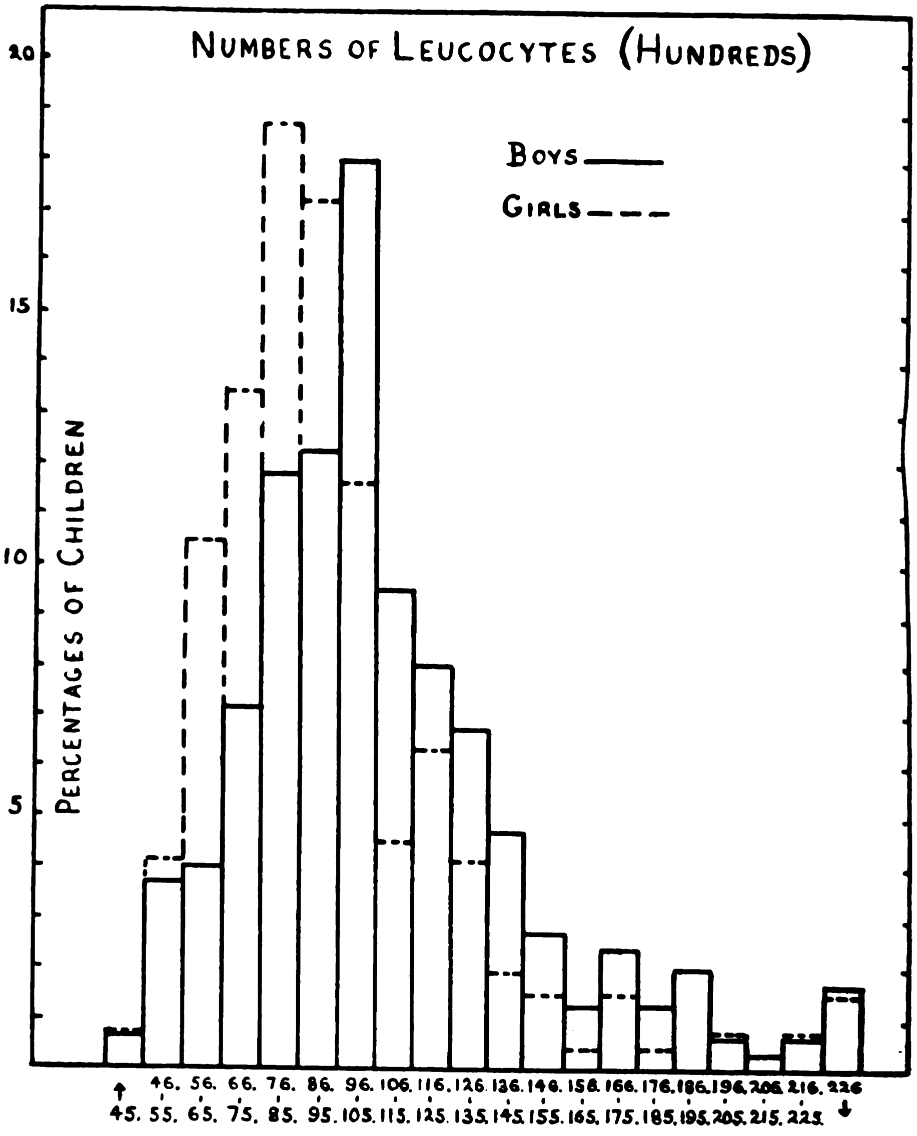


CHART II.

It is well known that the number of leucocytes in healthy children does not show the same degree of constancy as in adults, since the leucopoetic apparatus in the child is more labile, reacting to any irritation with much greater vigour. Besides, the technique of counting leucocytes is subject to a greater margin of error than that of counting red cells.

HAEMOGLOBIN

In the literature at our disposal we were unable to find any figures for the haemoglobin value in children which might be compared with our averages, since most observers have drawn their conclusions from a limited number of examinations only.

Leichtenstern (1909) gives as the normal average figure for children of ages between 11 and 15, 11·701 grams per 100 c.cm. blood; according to Perlin's (1903) estimation, using the Fleischl-Miescher apparatus, the haemoglobin value for children of ages ranging from 11 to 16 years varied between 78 and 88 per cent.

Chamberlain (1911) collected 1,433 haemoglobin estimations performed on 702 American soldiers in the Philippines; their average haemoglobin value was 89·6 per cent. It is clear, therefore, that 89 per cent., the average of 311 haemoglobin estimations (compare Table 2), corresponds to normal conditions for children.

The average figures for boys between the ages of 7 and 12 are fairly constant; there is a distinct and sudden rise in the haemoglobin value after the twelfth year, amounting to about 5 per cent.

In Chart 3 are plotted out, in an analogous way as previously done for erythrocytes, the range of the haemoglobin values against the number of boys in percentages. The percentages of haemoglobin are calculated on the understanding that 13·8 grams per 100 c.cm. represents 100 per cent. The graph shows that 53·4 per cent. of the 311 boys have a haemoglobin value exceeding 90 per cent., 19·3 between 86 and 90 per cent., and 27·3 below 86 per cent.

Girls.

In all, 269 erythrocyte counts and 263 haemoglobin estimations were performed on 269 girls of ages between 7 and 15 years. In Table 4 the average figures for the number of red and white blood corpuscles and the haemoglobin value are given.

TABLE 4. Average figures of blood counts and haemoglobin estimations of 269 school girls, according to age

Age	Average number of erythrocytes	Number of estimations	Average number of leucocytes	Number of estimations	HAEMOGLOBIN			Number of estimations
					Average absolute content	Average percentage	Average colour index	
7 years ...	4,774,800	9	8,048	9	11·84	86%	0·90	9
8 „ ...	4,833,800	7	8,711	8	11·15	81	0·85	8
9 „ ...	5,184,500	26	10,052	26	12·28	89	0·86	24
10 „ ...	5,092,400	35	9,510	34	12·68	91	0·90	31
11 „ ...	5,000,100	32	8,017	31	12·48	90	0·90	31
12 „ ...	5,161,500	47	7,204	45	12·65	91	0·89	36
13 „ ...	5,154,500	58	9,385	57	12·86	93	0·90	58
14 „ ...	5,116,800	46	9,067	45	12·98	94	0·91	44
15 „ ...	5,246,300	9	8,720	10	13·48	98	0·93	10
Total average...	5,107,700	269	9,077	267	12·66	92%	0·90	263

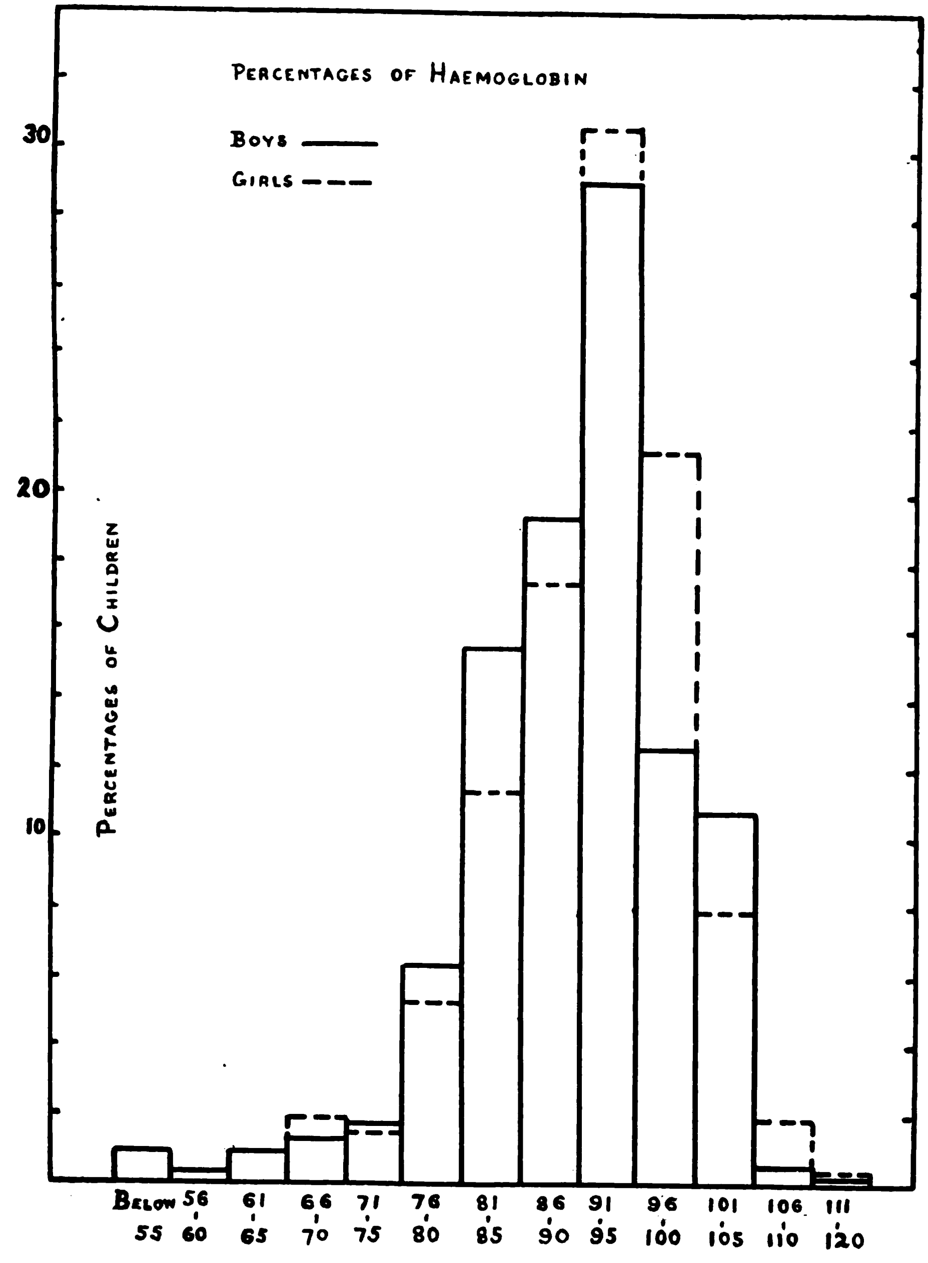

Percentages of Haemoglobin
Boys
Girls
Percentages of Children
30
20
10
Below 55
56 60
61 65
66 70
71 75
76 80
81 85
86 90
91 95
96 100
101 105
106 110
111 120

Chart III.

The number of erythrocytes of the youngest girls, 7 and 8 years of age, is comparatively low. Out of sixteen examined only five reached the standard of five millions. The averages of the erythrocyte counts of the girls between the ages of 9 and 15 are slightly higher and remarkably constant. The average for the whole number is 5,107,700. In fig. 1 the results of the individual counts are charted in the same way as for boys. Of the total number of girls, 1·8 per cent. had a red cell count below four millions, 44·8 per cent. between four and five, 46·3 per cent. between five and six, and 6·3 per cent. about six millions, altogether 64·1 per cent. between 4·5 and 5·5 millions.

The length of residence in tropical Queensland, in the same way as in the case of boys, does not seem to influence the number of erythrocytes, as the average count for eighteen girls who had lived in North Queensland for a shorter period than three years was 5,079,400.

The average figures of the blood counts performed during the summer months are practically the same as during the cooler season (compare Table 5).

TABLE 5.—Comparison of the average number of erythrocytes and haemoglobin value of 269 schoolgirls (263 resp.) made during the wet and dry season.

Season	Average erythrocyte count	Number of estimations	HAEMOGLOBIN			
			Average absolute content	Average percentage	Average colour index	Number of estimations
Wet season (hot)	5,062,000	179	12·66	92%	0·91	173
Dry season (cool)	5,198,500	90	12·67	92	0·88	90

No striking difference could be noted between the number of red blood corpuscles in dark and fair children; 178 counts performed on fair children showed an average of 5,093,300, 33 counts on dark children gave an average of 5,033,400.

The average number of white blood corpuscles of 267 leucocyte counts is 9,077. In Chart 2 the results of the individual counts are charted, indicating that out of the total number only 23·6 per cent. ranged higher than 10,000 per c.mm.

The average figures obtained for the haemoglobin of the different groups of girls are given in Table 4; on the whole, these increase with the age. Not too much weight can be placed on the low haemoglobin percentages of girls 7 and 8 years old, since the averages are based on numbers too small (eight and nine respectively) to allow a comparison with figures of other ages. The average of the haemoglobin value for the total number of girls is 92 per cent.

From Chart 3, showing graphically the results of the individual counts, it is evident that 20·1 per cent. of the children have a haemoglobin value below 86 per cent., 17·8 per cent. range between 86 and 90 per cent., in 62·1 per cent. more than 90 per cent. haemoglobin was found.

When we compare the results of the blood counts and haemoglobin estimations obtained in boys with those in girls (Table 6), it is evident that there are no marked differences in the average number of red blood corpuscles in both sexes.

TABLE 6.—Comparison of the average number of erythrocytes and haemoglobin value of 305 schoolboys and 269 schoolgirls of Townsville.

Average erythrocyte count	Number of blood counts	Average number of leucocytes	Number of estimations	HAEMOGLOBIN			Number of estimations
				Average absolute content	Average percentage	Average colour index	
5,046,100	305 boys	10,868	296	12·31	89%	0·88	311
5,107,700	269 girls	9,077	267	12·66	92	0·90	263
5,076,900	574 children	10,036	563	12·485	90·5	89	574

The average number for leucocytes in boys is distinctly higher than in girls.

The average for the haemoglobin value is higher in girls than in boys at all ages, and is even higher than the normal average figure given by previous workers.

SUMMARY

The examination of the blood of 574 school children who had spent all or in a few instances the greater part of their life in Townsville, situated in the coastal belt of North Queensland, tends to show that there is no striking difference in the number of erythrocytes and their colour index when compared with the averages obtained in temperate climates.

The number of observations is sufficiently large to warrant this conclusion, since any changes of a general nature would necessarily have influenced the final results.

Most conclusions of previous observers on the blood condition of Europeans in the tropics are based on examinations of individuals who had been living in a tropical climate for a comparatively short time only. Our observations, on the other hand, were carried out on children of European descent born in tropical Queensland, and furnish therefore additional and more conclusive evidence for the conception that a tropical climate as such does not bring about any marked change in the number of red blood corpuscles and their haemoglobin content.

PULSE RATE

Opportunity was taken to investigate, at the same time as the blood, the pulse rate and the blood pressure in the children of both sexes.

The figures of the pulse rate were, on the whole, far from normal; the majority of the children were naturally excited, and hence their pulse rate was more or less increased. Repeated determination of the pulse rate of a number of children showed great discrepancies, and the results had to be disregarded as inconclusive.

SYSTOLIC BLOOD PRESSURE

The systolic blood pressure was estimated by Martin's modification of the Riva Rocci sphygmomanometer, which had an armlet of 13·5 cm. breadth; the children were standing up. The results tabulated in Table 7 indicate an increase of the blood pressure with the rising age. The average systolic blood pressure of the girls is slightly higher than that of the boys, being 114 mm. mercury as compared with 111 mm., too small a difference to be taken into consideration.

TABLE 7.—Average blood pressure of school boys and girls, according to ages.

SYSTOLIC BLOOD PRESSURE

Boys			Girls		
Age	mm. Mercury	No. of estimations	Age	mm. Mercury	No. of estimations
7	112	10	7	103	9
8	109	28	8	110	8
9	111	38	9	110	26
10	111	48	10	110	36
11	112	38	11	117	33
12	112	50	12	116	47
13	113	44	13	116	59
14	113	37	14	116	46
15–16	114	6	15	120	10
Total average	111	299		114	274

Oppenheimer and Bauchwitz (1909) give as the average for the systolic blood pressure of children 12 years of age living in a temperate climate 112 mm. mercury, and Hensen (1906) found 116 mm. mercury as the normal blood pressure of children from 3 to 15 years of age.

The comparison of the blood pressure of North Queensland children with that of normal Europeans makes it evident that climate, as such, had no marked influence on the blood pressure in children. The fact that no seasonal variations were found, furnishes additional evidence on this point.

Chamberlain's figures, based on 6,847 estimations of the systolic blood pressure in 1,489 American soldiers in the Philippines, lead him to a similar conclusion, namely, that 'the blood pressure of Americans residing in the Philippines differs but little, if any, from the average at home.'

CONCLUSIONS

Careful blood examinations were performed on 574 school children in Townsville, of European descent, of ages between 7 and 15, of whom the majority had been born and had resided in tropical Queensland during their whole life.

The results indicate:

1. That the average number of red blood corpuscles is not diminished when compared with analogous figures for children born and bred in a temperate climate.
2. That the average haemoglobin content of the blood is normal.
3. That the number of leucocytes is slightly increased.
4. That the average blood pressure does not show any difference from that of normal children in temperate climes.

REFERENCES

Arneth (1904). Die neutrophilen weissen Blutkörperchen. Jena.

Chamberlain (1911). The red blood corpuscles and the haemoglobin of healthy adult American males residing in the Philippines. The Philippine Journal of Science, Vol. VI, No. 6, p. 483.

Cuthbert (1911). A comparison between bloods of some non-malarial and malarial European soldiers in Sierra Leone. Journal of Royal Army Medical Corps, July, p. 56.

Ehrlich and Lazarus (1910). Anaemia. London.

Eijkman (1891). Blutuntersuchungen in den Tropen. Virchow's Archiv., Vol. 126, p. 113.

Glogner (1892). Blutuntersuchungen in den Tropen. Virchow's Archiv., Vol. 128, pp. 160, 180.

Hensen (1906). Quoted by Vierordt, Anatomische Daten und Tabellen, p. 242.

Leichtenstern (1909). Quoted in Sahli's Klinische Untersuchungsmethoden, p. 839.

Marestang (1889). (*a*) Haematimetrie normale de l'European dans les pays chauds. Arch. de Med. Navale, No. 2. (*b*) Over tropische Anaemie. Geneesk Tijdsch v. Nederl. Ind., Vol. XXX, 1890.

Oppenheimer und Bauchwitz (1909). Arch. f. Kinderheilkunde, Bd. 42, p. 415. Quoted by Sahli, Klinische Untersuchungsmethoden, p. 164.

Perlin, A. (1903). Sahli. Loc. cit., p. 840.

Plehn (1892). Beitrag zur Pathologie der Tropen. Virchow's Archiv., Vol. 129, p. 285.

Sahli (1909). Klinische Untersuchungsmethoden.

Taylor, F. H., and Young, W. J. (1914). Journal of Tropical Medicine and Hygiene, XVII, pp. 225-227.

van der Scheer (1890). Over 200 genamde Tropische Anaemie. Geneesk Tijdsche v. Nederl. Ind., XXX.

Wickline (1908). Effects of tropical climate on White Race. Mil. Surgeon, 23, p. 283. (Quoted by Chamberlain.)

ON THE MIGRATION OF THE LARVAE OF *ONCHOCERCA GIBSONI* THROUGH THE CAPSULE OF THE WORM NODULE

BY
WILLIAM NICOLL, M.A., D.Sc., M.D.,
AUSTRALIAN INSTITUTE OF TROPICAL MEDICINE, TOWNSVILLE, QUEENSLAND

(*Received for publication* 4 *September*, 1914)

The life-history and the mode of transmission of the parasitic Nematode which causes worm nodules in cattle still await elucidation. Many observations have been made and a considerable amount of experimental work has been carried out, almost entirely in Australia, but so far the results obtained have hardly been commensurate with the amount of labour expended. The morphology of the worm itself, and the structure of the fibrous nodule in which it is invested, may be regarded as fairly exhaustively known. How and where the parasite enters its host, how it reaches its destination, and by what means the larvae are transmitted to another host, are questions on which we have but scattered items of information. The great majority of transmission experiments have had negative results, and there are very few positive observations at our disposal on which to base any manner of feasible hypothesis.

An excellent summary of the results obtained up till 1911 has been compiled by Harvey Johnston (1911). In that paper an account is given of the various lines along which experimental researches have been conducted, and of the few hypotheses which have been formulated. The observations and suggestions of previous workers are carefully examined and criticised, and the conclusion is arrived at that, even in spite of serious objections, the most likely transmitting agents are to be found amongst the mosquitoes, the true lice or a cattle fly (*Musca* sp.).

Since Johnston's review four further important contributions have been made on the subject. The first of these was by Cleland (1912) in Sydney; the second by Gilruth and Sweet (1912); the third by Breinl (1913) in North Queensland, and the most recent by Cleland (1914). Cleland made the somewhat unexpected discovery that three calves, born and bred on Milson Island, Hawkesbury River, New South Wales, had become infected with *Onchocerca* nodules. As these calves had not at any time been removed from the island, it is evident that infection must have occurred there, and that therefore all the factors concerned in the transmission of the parasite are present on the island. These important discoveries, though not immediately furthering our knowledge of the mode of transmission, cannot fail to help in materially lessening the number of possibilities. Thus, for instance, Cleland notes that the mothers of these calves were not infected, a fact which excludes the possibility of direct infection from mother to foetus or calf. The absence of leeches and ticks is also commented upon, so that they may apparently be definitely excluded from any participation in the life-history of the parasite. Another point of some significance is the absence of areas of bog and marsh on the island. This appears to preclude the possibility of the infection being water-borne, either directly or by the intervention of aquatic animals. The presence of a single deep water-hole on the island does not, in Cleland's opinion, invalidate this conclusion, as he does not consider it a likely or suitable place for infection to take place. This, however, cannot be admitted without some definite proof, and in view of Breinl's observations, which will be mentioned presently, the possibility of water-borne infection cannot be absolutely excluded.

Cleland's summary of the remaining possibilities differs somewhat from Johnston's. While including lice and mosquitoes, he does not mention the cattle-fly, but adds *Stomoxys calcitrans* as the most likely intermediary, with Mallophaga, Tabanidae and the sand-fly *Culicoides molestus*. He evidently attaches most importance to *Stomoxys* and mosquitoes.

Gilruth and Sweet's paper consists almost entirely of negative evidence, but they have made several observations which may prove of value in later work. They assert, for instance, that fresh infection rarely, if ever, occurs in cattle more than two years old. They also note the occurrence of a very large percentage of degenerate nodules. This may possibly explain the negative results obtained in many experiments to determine the mode of transmission of the larvae. Gilruth and Sweet dismiss the possibility of direct infection or of infection from the soil, and they also appear to abandon the idea which they formerly entertained of lice being the intermediate hosts. Like Cleland, they come to the conclusion that a biting fly is the most probable intermediary.

Breinl's observations are of the first importance, as they are amongst the few definitely positive results which have been obtained in any direction. They confirm a suspicion which had been hinted at by previous observers, namely, that the larvae could make their escape from the worm nodule, pierce the intact skin of their host, and so find their way to the exterior. Breinl's discovery is of interest from the fact that it is the first record of such an occurrence in the life of any parasite of this class. Although it is true that the event was only observed in a few cases in the course of a large number of experiments, still it cannot be denied that even these few positive observations are sufficient to establish the fact that the *Onchocerca* larvae can make their escape through the unbroken skin of their host either naturally or under experimental conditions. The only objection which can be urged against this statement is that some slight abrasion of the skin may have been caused in the act of shaving or scraping. So far as appears, however, stringent precautions were taken that this should not occur.

Cleland's latest paper (1914) is a comprehensive account of an extended series of experiments directed on the one hand towards ascertaining the path taken by the worm in the infected animal, and on the other towards incriminating biting flies or mosquitoes as the intermediate host. He has elucidated several interesting points, and has made the case in favour of *Stomoxys calcitrans* a very strong one. His feeding experiments with *Stomoxys* are not by any means conclusive, but they show that the larvae can live in it for at least as long as they live in water and much longer than in non-biting flies. Of more importance is his discovery of the worms in their passage up the hind leg of the infected animals. His suggestion that the formation of nodules is an accident in the life history of the worm and that larvae are chiefly produced while the worm is free is not a new one, and appears not altogether necessary in view of the experiments to be detailed later. It shows, however, that it is not necessarily in the vicinity of the nodule that larvae are to be found, but that they may be met with in the subcutaneous tissues over a considerable extent of the body.

It was with the view of controlling and confirming these observations that the present investigations were undertaken. In the first instance a series of over thirty experiments, similar to those of Breinl, were carried out. Two moderately infected, middle-aged, cows were used. Each had about half-a-dozen palpable nodules varying in size from 15 mm. to 30 mm. in diameter. Each of the nodules were selected in turn for experiment. In most cases the skin was previously depilated with a mixture of barium sulphide, zinc oxide and starch, but in some instances the hair was not removed.

The experiments were started in March, 1913, and continued till the end of July. The wet season was just ending, though there were several rainy days during the course of the investigations. The experiments were started at different hours of the day, from 8 a.m. till 6 p.m., on the chance of there being some particularly favourable time for the escape of the larvae. Various other modifications were also adopted. In every case the water used was tap-water previously boiled, filtered, and allowed to cool to body-temperature. In some of the early experiments the skin over the nodule was washed with absolute alcohol and then with sterile water, but in the later experiments the alcohol was omitted.

In the first nine experiments a calico bandage folded to form a square pad was soaked in sterilized water, covered with oiled silk, and strapped over the selected area. This was left on for one-and-a-half to three hours, after which the pad was carefully washed in sterile water and wrung out and the skin scraped with a blunt scalpel. In the later experiments a small circular vessel of three inches diameter was substituted for the calico pad. This was filled with sterile water and applied continuously to the selected part.

On the few occasions which offered, the cow was exposed to the rain for two or three hours before the experiment was started, in the expectation that rain might have some influence in stimulating the egress of the larvae.

Notwithstanding the various modifications adopted, not one of the experiments gave a positive result. In no case were any larvae observed, either in the water or in the scrapings from the skin, although a very exhaustive examination was made on every occasion. These negative observations do not vitiate the validity of Breinl's results, but they show that the exodus of the larvae does not follow simply on the application of water to the skin over every or any nodule. Evidently some other factor or factors are essential. These may be associated with climatic or environmental conditions, or may be inherent in the condition of the nodules themselves. It may be, as will be gathered from what follows, that none of the nodules in either of the two cows used were in a suitable state, i.e., for the emigration of the larvae.

Further experiments were conducted with nodules excised from the carcases of cattle slaughtered at the meat works, the object being to ascertain whether the larvae are in the habit of penetrating the capsule of the nodule in large numbers or if the occurrence is only occasional.

In the first experiment two freshly excised nodules were used. One measured about one inch in diameter, the other half an inch. The first was surrounded by a mass of muscle, the second by a small amount of connective tissue. From the first nodule the muscle was carefully cut away and divided into two portions. The two nodules and the two pieces of muscle were placed in separate, covered, glass capsules, nearly filled with filtered rain-water. The capsule containing the larger nodule and that containing one piece of muscle were placed in the incubator at 37° C. The other two were allowed to remain at room temperature (21° to 26° C.). At the end of an hour the water was poured off from each of the capsules, which were cleaned out and filled with fresh water. This was poured off at the end of another hour and a half, the capsule again carefully cleaned, and fresh water added. The same process was repeated three times during the ensuing seven hours and once on each of the following three days. The fluid poured off from each capsule was centrifugalised and the centrifugalate examined for larvae.

The larger nodule gave a negative result throughout, as did the piece of muscle which had been left at room temperature. The other piece of muscle showed one larva on the second examination (i.e., at the end of two and a half hours). The smaller nodule showed two larvae at the end of six hours, and an additional one was found at the expiry of another three hours. No larvae were found later than this.

In the second experiment eight nodules (nodules 5-12) were used. It was similar to the first, but two nodules were placed in acidulated water (1% HCl.) and two were placed in alkaline water (1% NH_3). In the case of the other four, ordinary filtered rain-water was used. Two cross-shaped incisions about 1/16th of an inch deep were made in the capsule of nodule No. 12. Half of the capsules were placed in the incubator at 37° C., the others left at room temperature. The same procedure was adopted as in the first experiment. The results are tabulated below.

Experiment started at 1 p.m., 30th July.

	30th July			31st July	1st August
	2.15 p.m.	5.15 p.m.	9 p.m.	9 a.m.	12 noon
5. Acidulated water, 37° C.	4	0	2	0	0
6. Acidulated water Room temperature	0	3	0	0	0
7. Ammoniated water, 37° C.	2	2	0	6	1
8. Ammoniated water Room temperature	2	12	0	0	0
9. 37° C.	42	8	0	0	3
10. Room temperature	439	2,000	Very numerous	30	7
11. 37° C.	47	18	0	0	0
12. Room temperature	42	230	20	14	0

At the end of the experiment the nodules were cut open and their contents examined. In numbers 5 and 6 the worm appeared as a caseous mass. In numbers 7 and 8 the worm was in good condition but the larvae were all dead. A similar condition was found in 10 and 12, but in 9 and 11 the interior of the nodule was in a state of putrefaction.

From this experiment it would appear that increase of temperature does not notably, if at all, favour the escape of the larvae from the capsule. It would also appear that acid and ammonia, at least in the strength used, have a decidedly retarding effect.

It may be mentioned that all the nodules were carefully examined before the experiment to ensure that no cut or injury was present in the capsule. The result in the case of No. 10 was so unexpected that the nodule was subjected to an especially careful examination after the experiment, but no sign of any cut or other perforation could be detected. The capsule was perfectly intact, and was surrounded by a considerable amount of connective tissue.

At the same time as the above experiment was being conducted a number of smaller nodules surrounded by fibrous tissue were placed in two glass vessels with water and kept at room temperature. These were examined after seventeen hours, when it was found that 101 larvae had made their escape in one case and twelve in the other. After another twelve hours a second examination showed eight in one and twenty-two in the other. A third examination fifteen hours later proved entirely negative.

This experiment appears to indicate that in many *Onchocerca* nodules, if not in all, larvae are constantly present in the capsule, and are continually migrating through it in greater or less numbers into the surrounding tissues.

A third series of experiments was started on the 12th August, at 11.30 a.m. Examinations were made at intervals throughout this and the following two days. The procedure was identical with that in the preceding series, but all the nodules were kept at room temperature. At each period of examination one nodule was transferred to Zenker's fluid, fixed and prepared for sectioning. At 3 o'clock on the same day a fourth series of experiments was started, and these were examined at the same intervals as the others.

The results of these experiments are set forth in the following table:

	12th August		13th August			14th August	
	2 p.m.	5 p.m.	9 a.m.	12 noon	4 p.m.	9 a.m.	2 p.m.
15	2	3	0	0	0	1	—
16	2	2	0	1	0	0	0
17	0	0	0	0	0 (fixed)		
18	3	2	1	0	1	0	0
19	0	0	5	0	0	0	0
20	1	0	2	0	0	0	0
21	0 (fixed)						
22	0	5 (fixed at 3 p.m.)					
23	0	2 (fixed at 6 p.m.)					
24	2	0	0 (fixed)				
25	0	2	3	1	2 (fixed at 3 p.m.)		
26	Started at 3 p.m.	0	1	0	0	0	0
27		1	0	0	1	0	0
28		0	0	1	0	0	0
29		1	34	42	45	6 (fixed)	
30		0	2	0	1	1	0

From the above table it will be seen that fewer larvae were liberated from the nodules in these experiments than in the previous series. Only in No. 29 was there anything approaching a free production. It will also be noticed that in the case of these nodules the larvae only began to make their escape in any numbers several hours after the experiments had been started. This might be taken to indicate that the water in which the nodule was immersed had a distinct, though slow, effect in stimulating the migration of the larvae through the capsule.

The result in No. 15 is of interest, for in this case a period of at least twenty-three hours elapsed during which no larvae made their escape. In No. 27 a similar period of at least nineteen hours occurred. In both cases a single larva made its appearance some time later. No. 17 was entirely negative, and when sections from it were examined, absolutely no larvae were found in the capsule, though in the centre of the nodule there was a small mature female worm.

In the sections from No. 21 there were very numerous larvae throughout the capsule. They were arranged roughly in three concentric strata. These corresponded practically to the three densest layers of the capsule. There were extremely few in the looser tissue, and none at the periphery of the capsule. In the sections from Nos. 22 and 23 there was a considerable number of larvae scattered irregularly throughout. No larvae were found in No. 24, but there were several in No. 25. In No. 29 a concentric arrangement of the larvae could be made out, but not so distinctly as in No. 21. Some of the adherent loose connective tissue was removed from this nodule and cut into sections, but no larvae could be detected in it.

A series of experiments similar to the foregoing was started on October 13th. At the beginning, six nodules were fixed and prepared for sectioning. Others were fixed at varying intervals.

The results of the experiments are as follows:

Started 1 p.m.	13th October			14th October			15th October	
	3.30 p.m.	5.15 p.m.	9 p.m.	9 a.m.	12.30 p.m.	5.30 p.m.	11 a.m.	3.30 p.m.
32	40 (fixed)							
33	—	5 (fixed)						
34	30	0	16 (fixed)					
35	—	36	40	12	—	2	0	0
36	6	—	10	4 (fixed)				
	—	4	12	0	—	1	0	0
38	1	—	2	4	0 (fixed)			
40	4	—	12	0	—	3 (fixed)		
41	—	0	0	0	—	0	0	0

These results are rather similar to those obtained in the previous series. The longest time during which larvae continued to escape from any of the nodules was twenty-eight hours in the case of Nos. 35, 37 and 40. These three nodules were cut open at the end of the experiment and the larvae inside the parent worm were found to be all dead. No. 41 was entirely negative throughout.

Examination of the sections made from nodule 31, which had not been immersed in water, showed that the capsule was entirely devoid of larvae. Three series of sections from different parts of the capsule were made and a large number of sections carefully gone through, but no trace of larvae could be detected. A considerable amount of effused blood was present in the capsule, so that the nodule had apparently been recently bruised.

In the sections from nodule 32 only a very few larvae were present. In No. 33 they were more numerous, but still comparatively scanty. The sections from No. 34 were rather interesting. The inner layers of the capsule showed an extremely large number of larvae, but in the outer layers there were absolutely none at all. One might infer from this that the influence of the water had caused all the larvae already in the capsule to escape, and had induced a fresh migration from the interior of the nodule. In the case of Nos. 32 and 33 it might be supposed that the action of the water had not been allowed to continue for a sufficiently long period.

The foregoing inference receives support from the fact that in nodule 36, which had been subjected to the action of the water for twelve hours longer than No. 34, an almost exactly similar condition was found, with the difference that a number of larvae were met with in the median layers and a few in the outer layers. In nodule 38, however, the result was discordant, as only a few larvae were seen, and there almost entirely in the outer layers of the capsule. This might be explained by the death of the adult worm. In No. 40 the result was somewhat similar to that in Nos. 34 and 36, and here again the larvae were much more numerous in the walls of the capsule even than in No. 36.

Of the other nodules which had not been immersed in water, two showed numerous larvae in the walls of the capsule, one was entirely negative, while the others had a few.

On November 13th a series of experiments somewhat different from the foregoing was started. The following are the details. Nodules were immersed in water continuously for varying lengths of time. The nodules were then fixed and the water examined for larvae.

					Result
44	fixed	in formalin	immediately		
45	„	„	after 1	hour in water	No Larvae
46	„	„	„ 2	„ „	40 „
47	„	„	„ 3	„ „	12 „
50	„	„	„ 6	„ „	12 „
48	„	„	„ 18	„ „	No „
49	„	„	„ 24	„ „	No „
51	„	„	„ 24	„ „	No „

It must be noted that nodules 48, 49 and 51 were kept in the ice chest for a few hours previous to the experiment, and this had probably been sufficient to kill all the larvae.

On examination of the serial sections no larvae were found in the capsule of No. 44, and only a few in No. 45. In No. 46, however, the larvae were very numerous. In 47, 48 and 50 they were extremely scanty, while in 49 and 51 they were entirely absent.

The result of this experiment is not at all conclusive, yet it shows that in the case of a suitable nodule, such as No. 46, a considerable number of larvae may make their escape from the capsule in quite a short space of time.

In all the foregoing experiments attempts were made to keep the larvae which had emerged from the nodules alive in water for as long as possible. In the most successful case they remained alive for only seventeen hours. Gilruth and Sweet (1911) and Breinl have made similar observations, but with larvae removed mechanically from the interior of the nodule. They found that the life of these larvae in water could not be prolonged beyond two days.

SUMMARY AND CONCLUSIONS

The results of these experiments, while not confirming Breinl's observations, go to show that *Onchocerca* larvae can and do make their escape through the capsule of the worm nodule. They usually do so in small numbers, but may at times, or in some cases, migrate in comparatively large numbers.

It is evident that the results of Breinl's work form a strong argument in favour of water-borne infection. Against this, however, must be placed two very weighty facts, namely, that the larvae are not adapted for independent existence in water, and that their life in that medium, so far as observed, does not extend beyond two days. Notwithstanding these objections, water-borne infection, direct or by intermediary host, must be considered as a possibility.

Taking everything into consideration, insect-borne infection appears to present the greatest measure of probability. Leiper (1911) supported this view on purely morphological grounds. Anatomically the adult worm is so closely allied to the *Filariae* that there is some justification in expecting an analogous life-history. On the other hand the habitat of the worm is different and approaches more closely that of the Guinea-worm (*Dracunculus*). This fact has not failed to lend some bias in favour of water-borne infection.

Practically all observations go to show that this difference in habitat from that of the *Filariae* is correlated with a difference in the life-history. De Does (1904) is reported to have found *Onchocerca* larvae in the blood, but no other observer has succeeded in doing so. It has, again, been suggested that the larvae may circulate in the blood like *Microfilariae* before the fibrous capsule is formed around the adult, or that they may only occur in the blood of young cattle. Both of these seem quite unnecessary hypotheses in view of the fact that the larvae are in the habit of migrating through the capsule of the nodule into the surrounding tissues, and eventually finding their way into, or even through, the cutaneous surface.

As has been shown in the foregoing notes, the number of larvae so liberated may, in certain cases, be very considerable, and quite sufficient to ensure a moderate chance of a few being taken up by a blood-sucking or biting insect. If it be the case that the larvae are in the habit of emerging through the skin of their own accord there is the possibility of some non-biting insect becoming infected, but in such a case it would be difficult to understand how re-inoculation could be effected.

REFERENCES

BREINL, A. (1913). The Life-History of *Onchocerca gibsoni*. Australian Institute of Tropical Medicine Report for the year 1911, pp. 14-17.

CLELAND, J. B. (1912). Some Notes and Suggestions in Connection with the Etiology of Bovine Onchocerciasis. The Australasian Medical Gazette, 1912. April 27th, p. 4.

—— —— (1914). Further Investigations into the Etiology of Worm Nodules in Cattle. Dept. of Trade and Customs, Commonwealth of Australia.

DEDOES, J. (1904). Worm Fibromen en Filaria—embryoen in het bloed. Geneeskundig Tijd. v. Ned. Ind. Deel., XLIV, pp. 15. (Quoted from Johnston, 1911.)

GILRUTH, J. A. and SWEET, G. (1911). *Onchocerca gibsoni*: the cause of worm nodules in Australian Cattle. Commonwealth of Australia Report.

—— —— (1912). Further observations on *Onchocerca gibsoni*, the cause of worm nodules in Cattle. Proc. Roy. Soc. Victoria, XXV (new series), pp. 23-30.

JOHNSTON, T. H. (1911). On the occurrence of worm nodules in cattle—A Summary. Proc. Roy. Soc. Queensland, XXIII, pp. 207-231.

LEIPER, R. T. (1911). On Onchocerciasis in Cattle with special reference to the structure and bionomic characters of the parasite. Report of the Local Government Board on Public Health and Medical Subjects. New Series, No. 45.

THE MORPHOLOGY, BIOLOGY AND ECONOMIC IMPORTANCE OF *NOSEMA BOMBI*, N.SP., PARASITIC IN VARIOUS HUMBLE BEES *(BOMBUS* SPP.*)*

BY

H. B. FANTHAM, D.Sc. (LOND.), B.A. (CANTAB.)

LECTURER ON PARASITOLOGY, LIVERPOOL SCHOOL OF TROPICAL MEDICINE,

AND

ANNIE PORTER, D.Sc. (LOND.), F.L.S.

BEIT MEMORIAL RESEARCH FELLOW, QUICK LABORATORY, CAMBRIDGE.

(Received for publication 26 *October,* 1914)

PLATE XXX

CONTENTS

I. INTRODUCTION 623
II. THE HOSTS OF *Nosema bombi* 624
III. THE EFFECTS OF *Nosema bombi* 624
IV. LIFE HISTORY OF *Nosema bombi* 626
V. METHODS 627
VI. MORPHOLOGY 628
The Planont or Amoebula 628
The Meronts and Merogony 628
Spores and Sporogony 629
VII. MODE OF INFECTION 632
VIII. THE ECONOMIC IMPORTANCE OF *Nosema bombi* 633
IX. SUGGESTED PREVENTIVE MEASURES 634
X. SUMMARY 636
REFERENCES 637
EXPLANATION OF PLATE 638

I. INTRODUCTION

Two lines of research have recently been much to the fore in connection with parasitic Protozoa. The first line of work deals with the Protozoa whose effects on man, either direct or indirect, are obvious. Such are the investigations on the trypanosomes producing sleeping sickness in man and on the Piroplasma causing red water and consequent great losses in cattle.

The second line of research is one that is now becoming increasingly important, though its value has only more recently begun to be appreciated. Among such researches may be included those on the natural flagellates of insects, for example, *Herpetomonas jaculum* and *Crithidia gerridis*, and the use of elucidating their life-cycles in the investigation of diseases like kala-azar. Again, the importance of a small Microsporidium, *Nosema apis*, in producing heavy mortality among hive bees in Great Britain, has been very prominent, and has been impressed on the public at large since it produces the so-called Isle of Wight bee disease. An allied organism *Nosema bombi*, n. sp., is parasitic in, and pathogenic to, humble (or bumble) bees, as we have discovered, and its life-cycle is set forth in this paper. While the effects of *N. bombi* at the moment may not be widely felt, it has already produced losses in certain districts by its ravages among the humble bees responsible for the fertilisation of the red clover crops. Subsequent financial loss has resulted from poorness of such crops and also paucity of seed.

II. THE HOSTS OF *NOSEMA BOMBI*

Nosema bombi is not restricted to one species of the genus *Bombus*, or even to that genus. During the last few years we have found the parasite in the following insects: *Bombus agrorum*, *B. hortorum*, *B. latreillelus*, *B. lapidarius*, *B. sylvarum*, *B. terrestris*, *Apis mellifica*, and imported *Apis florea*. Morphologically, the parasite presented the same facies in each of these hosts, while cross-infection experiments showed that its pathogenic properties were identical in each case, and that transplantation to another host did not result in differences of structure. It was therefore concluded that the one organism only, *Nosema bombi*, was present in each of the hosts, and that in each case we were dealing with the same organism.

III. EFFECTS ON THE HOSTS

From observations extending over some years, the conclusion has been reached that *Bombus agrorum* is the humble bee in which the parasite is most common. In certain districts practically every *B. agrorum* examined was found to be infected, but in other districts infection was rare. *B. terrestris* was also fairly often infected, while small numbers of the other hosts were found to be parasitised.

Infected humble bees frequently lose their power of flight, and crawl in a blundering manner over the ground. They seem very easily irritated, and sting on far less provocation than usual. On the extrusion of the sting, or on the abdomen of an infected *Bombus* being touched, a discharge of faecal material occurs in some cases, but this is far less obvious than when a hive bee suffering from the effect of the allied *Nosema apis* is similarly treated. Usually the infected humble bee dies as a result of the infection, and in bad epizootics numbers can be picked up dead on banks in which many have formed a nidus, each for itself.

Internally, the parasite is found mainly in the Malpighian tubules, and to a less extent in the alimentary canal of the bee, and there are considerable differences between infected and normal tubules (Pl. XXX, figs. 2, 3). The most heavily infected part is undoubtedly the Malpighian tubules (fig. 3), and in most cases the microsporidiosis is chiefly localised in the excretory system of the humble bee.

When an early infection is encountered, the chyle stomach alone may be parasitised, but the infection rapidly spreads to the Malpighian tubules. So rapid is the spread that humble bees artificially infected by way of their food have shown the commencement of Malpighian infection in forty-eight hours. The small intestine becomes infected after or simultaneously with the excretory tubules, but the infection of the gut is never so severe as it is in the case of a hive bee infected with *Nosema apis*.

The fat body of an infected humble bee, whether the insect be examined in summer or winter, is always much reduced in bulk compared with that of the normal humble bee. The ovaries of an infected queen are often smaller than those of a normal subject. The haemocoelic fluid appears to be more abundant in the diseased than in the healthy humble bee, perhaps as a result of the impairment of the function of the Malpighian tubules. These latter often have their lumen partly obliterated by masses of *Nosema* spores, while the multiplicative stages of the parasite reduce the general composition of the tube to an almost structureless, riddled mass.

Malpighian and intestinal infection with *Nosema bombi* co-exist frequently, but intestinal parasitism alone has not been encountered hitherto except in cases of very recently contracted infection. Intestinal infection seems to play a subsidiary part in bringing about the death of the parasitised host. Such characteristics are in marked contrast with those of *N. apis.*

The genital organs of both male and female *Bombi* do not seem to be affected directly by the parasite, and there has been no evidence of hereditary infection obtained up to the present.

IV. THE LIFE HISTORY OF *NOSEMA BOMBI*

A brief outline of the life-history of *Nosema bombi* may be of service, for purposes of clearness, before the morphology of the parasite is discussed in detail. The *Nosema* forms protective structures, termed spores, which serve as resistant forms, well adapted to life outside the host and capable of reproducing the organism with great rapidity when they gain access to a fresh host. The spores of *N. bombi* (figs. 34-42) are voided with the excrement of the humble bee, and thus are in a position to contaminate the food or drink of other insects. Assuming that such spores are swallowed by another humble bee, the sequence of events briefly is as follows: The spores remain relatively unchanged until they reach the chyle stomach of their host. Then the spore coat or sporocyst of each becomes softened, an anchoring polar filament is ejected and fixes the spore to the gut-wall, and the organism within creeps out as a small motile body known as the amoebula or planont (Pl. XXX, fig. 4). After a short period of motility, or wandering, the planont may divide into two, or without any such division it may penetrate one of the digestive cells (fig. 1), or a cell of a Malpighian tubule (fig. 3), and there enter upon the growing, feeding stage of existence. The trophozoite (figs. 5-13) thus formed loses its motility.

After growth has proceeded for some time, the parasite enters upon a period of multiplication known as schizogony or merogony, the merozoites formed being more often termed meronts. Two forms of merogony occur. The usual type is by repeated binary fission (figs. 14-23), a colony of individuals resulting therefrom.

Less commonly the trophozoite produces four merozoites within itself (figs. 24-31). The appearance of the merozoites varies with the space in which they are formed. The most common type is roughly oval.

Each uninucleate meront ultimately becomes a sporoblast giving rise to a single spore, important changes occurring within it before the spore is mature. Thus from one meront one sporoblast, and ultimately one spore, is produced, whereby the parasite is placed in the genus *Nosema* among the Microsporidia.

V. METHODS

It is necessary to describe the morphology and life-history of the parasite from the earliest stages, and for this purpose smear preparations have proved of more service than sections. The humble bee was dissected and the alimentary tract removed intact and placed in normal saline solution. Each Malpighian tubule could then be separated out as required and removed. The tubules were slit open with a very small knife made from a flattened needle, and smears made of them. The same treatment was adopted for the different portions of the alimentary canal, which were teased serially and smeared. Wet-fixation by osmic acid vapour, followed by absolute alcohol, by corrosive-acetic-alcohol, or by Bouin-Duboscq fluid, all were used. The smears were stained by iron-haematoxylin, glycerin-haematin, or combinations of these with erythrosin. Giemsa solution and another modified Romanowsky stain were sometimes used.

Sections also were employed, the entire gut of a humble bee, together with its attached Malpighian tubules, being sectioned serially. The fixation and staining methods adopted were broadly the same as for the smear preparations.

Throughout the research, extending over four years, much attention has been given to the study of the living organism, in order that the stages of development might be accurately traced, a procedure that is impossible when fixed and stained materials only are employed.

VI. MORPHOLOGY

The morphology varies with the stage of development, and hence may be dealt with along developmental lines.

The life-history commenced with the spore (Pl. XXX, figs. 34-42) which, when swallowed by a new host, softened and allowed an amoebula to escape, the amoebula or planont being the first stage in development.

The Planont or Amoebula

Each planont (fig. 4) is a very small organism, about 2·2 μ in diameter, capable of active movement by means of pseudopodia, one pseudopodium as a rule being protruded at a time. The planont as it leaves the spore is usually binucleate. Division occurs and two uninucleate planonts (fig. 4) are produced. The body cytoplasm of the planont is finely granular, and the nucleus may be relatively homogeneous or may show a karyosome. The motile period is relatively short, and the parasites soon penetrate a cell of the lining of the alimentary tract (fig. 1), or work their way to the Malpighian tubules of the host. There they become rounded (figs. 3, 5), and behave as ordinary trophozoites, growing and feeding at the expense of the surrounding medium formed by the host cell. After a time they enter upon the multiplicative phase, and are then known as schizonts or meronts.

The Meronts and Merogony

Meronts vary considerably in size and shape, according to the space available for their development. The simplest forms primarily are oval (figs. 6, 9) or round (fig. 12), but when division occurs, elongate forms (figs. 21, 31), heart-shaped (figs. 20, 22, 24), and even somewhat irregular ones (fig. 26) may be produced owing to mutual compression or to the pressure exerted by the limits of the host cell. The body of a large meront shows finely alveolar cytoplasm (figs. 23, 27-29). The nucleus may be vesicular with a karyosome (fig. 11), or have its chromatin in the form of relatively evenly distributed grains (figs. 6-9). Extra-nuclear chromatin is rarely present. Division of a meront is initiated by a simple division of the nucleus, of a promitotic type. There is concentration of the chromatin at opposite poles of the nucleus, and then gradually the ends diverge, a strand frequently connecting the two nuclei for some time (figs. 14, 15, 17). At other times the strand is very short, or may appear to be quite suppressed (fig. 16). The daughter meronts vary greatly in appearance—an elongate parent (fig. 21) may give rise to two practically oval daughter forms, while from a circular meront two elongate oval ones may be produced. The rate of division also varies. When a second division occurs, the nuclei may divide simultaneously (fig. 25) so that four nuclei are produced at one time (figs. 26-31), or one nucleus may precede the sister nucleus in division (fig. 24) whereby three new forms appear to arise simultaneously. Cytoplasmic cleavage always follows nuclear multiplication, and this fission may be long delayed, even, in fact, till spore-formation occurs. The result is that multinucleate meronts (figs. 26-33), sometimes inaptly known as plasmodial forms, are found co-existing with single, uninucleate individuals. They may be seen in heavy infections where space is very limited, and also are found sometimes in the spaces between intestinal cells, though they are rare.

The number of daughter meronts arising from any particular meront varies largely with the amount of space available, and with the quantity of nourishment that the host can provide. The number of meronts formed by *N. bombi* in the gut of its host does not seem to be so large as that produced by *N. apis* in the honey bee. However, the number of meronts produced in the Malpighian tubules (fig. 3) is relatively large, so that the total infection may be as great. When the host is becoming exhausted, the parasite is also adversely affected, and proceeds to adapt itself to the new conditions, and to assume a resistant form capable of life outside the host. The power of further multiplication is lost, each meront becomes a sporoblast, and ultimately gives rise to a single spore.

Spores and Sporogony

As with *Nosema apis*, complicated nuclear changes accompany the formation of a spore of *N. bombi* from a meront. Five nuclei are produced in all, but when three of these have fulfilled their function they gradually degenerate and disappear. Upon losing the power of producing more meronts, the secretion of a spore-coat or sporocyst, together with rapid nuclear multiplication, begins. The meront becomes the sporoblast, which gradually merges into the spore.

At first it is uninucleate (fig. 34). Rapid division of the nucleus into two occurs (fig. 35). One of the daughter nuclei gives rise by divisions to three nuclei, the other to two. One of the first series ultimately controls the polar capsule, and is called the polar capsule or capsulogenous nucleus. Of the remainder, one pair remains in the sporoplasm and forms the sporoplasmic nuclei or chromatin of the amoebula, while the members of the other pair pass to opposite edges of the spore. The separation of these nuclei is not easy to follow, but it closely adheres to the plan outlined by us in 1912 for the spore of *N. apis*. Intermediate stages are indicated in figs. 36, 37. The two peripheral nuclei regulate the rate of formation of the sporocyst, and are variously known as the sporocyst, valvular, or parietal nuclei. They usually are somewhat slender and elongate nuclei (figs. 38-40). All the nuclei are not easily visible at one time; some are much more prominent than others. They also are not easily seen, the wall of the spore constantly becoming more and more opaque, till, when it is mature, it is almost or quite impossible to determine the contents without the aid of powerful reagents.

While nuclear changes have proceeded apace, cytoplasmic ones also have occurred. Very early in spore-formation two vacuoles form, one at either end of the spore (figs. 34, 35). The cytoplasm (now termed sporoplasm) consequently becomes girdle-like in appearance (figs. 35, 36, 41, 42). Within one vacuole, the polar capsule, an elastic filament is formed which passes backwards and coils up in the opposite, posterior vacuole (fig. 40). This is the polar filament. The filament is capable of ejection through a weak spot or micropyle in the sporocyst (figs. 41, 42), and is stimulated naturally into ejection by the digestive juices of a new host. Artificially, ejection of the filament can be induced by the action of nitric acid, acetic acid, and some other substances, such as iodine solution or glycerine.

The spores vary somewhat in size, being on the average 5·2 μ long and 3·7 μ broad. Occasionally spores 6·5 μ to 7 μ in long diameter are found. They show less dissimilarity in size in any one bee than do those of *N. apis*. The younger humble bees seem, on the whole, to have forms of *N. bombi* that produce larger spores than are found in older bees. That there is but one parasite concerned is proved by cross-infection experiments. Old *Bombi* fed on the large strain of spores become infected, but the spores are often smaller. When such strains of small spores are fed to young *Bombi* or to hive bees, the larger form of spore reappears and predominates.

Nosema bombi of humble bees differs from *Nosema apis* of hive bees in several respects, some of which may be mentioned:—The planonts of *N. bombi* are larger than those of *N. apis*, and have greater powers of movement. The number of meronts produced by a single trophozoite of *N. bombi* is less than the number produced by a trophozoite of *N. apis*. Large multinucleate or plasmodial forms of *N. bombi* are rare, even in heavily infected humble bees. The nuclei of the meronts of *N. bombi* seem better defined than those of *N. apis*. The spore of *N. bombi* is somewhat flatter, and is less refractile than that of *N. apis*. The sporocyst of the former, as judged by its reactions with stains and chemical reagents, is less dense and thinner than that of *N. apis*. Hence the spore of *N. bombi* is less resistant to the shrinkage or slight distortion which may occur with some fixatives. As regards the size of the spores of the two species, there are many specimens of that stage of *N. apis* measuring 5 μ by 2 μ with typical oval, rice grain shape, while the average size of the spores of *N. bombi* measured by us is 5·2 μ by 3·7 μ. There is more variation in the size and shape of the spores of *N. apis* than in those of the parasite described in this memoir. Fresh spores of *N. bombi* usually appear the larger and broader of the two. Polymorphism is less well marked in the parasite of humble bees than in *N. apis*. Further, *N. bombi* is more restricted in its distribution within the host, the alimentary tract being usually but slightly parasitised and the main infection occurring in the Malpighian tubules. Lastly, among humble bees the queens and workers have been found to be infected with *N. bombi* in about equal proportions, a condition certainly not obtaining in hive bees infected with *N. apis*.

VII. MODE OF INFECTION

The mode of infection of the various species of *Bombus* with *Nosema bombi* is contaminative, and, as with *N. apis*, food and drink soiled with the excrement of the parasitised insects are the main sources of infection. *Bombus agrorum* constructs small mossy nests in which the brood is raised. The food cells from such a nest have been found to contain also *N. bombi* when examined microscopically. The nidus of *B. hortorum* dug out from a bank, and those of various other species obtained from meadows and removed from abandoned mouse-runs (a rather favourite site in some localities), have also yielded the parasites. Water that had collected in the hollow of a tree near a ditch was visited by many *B. latreillelus* and *B. agrorum*. On examination, a few drowned humble bees in various stages of decomposition and a quantity of *Nosema bombi* spores were found. This spot was for a short time a good collecting ground for obtaining moribund humble bees.

It should be mentioned that humble bees, like hive bees, can suffer and die from *Nosema apis*, contracted when robbing hives. Similarly, hive bees have been found dying from the effects of *N. bombi*, which has been present in them in their Malpighian tubules in the form of both meronts and spores. There are differences in refractivity, shape and size of the spores of these two species of *Nosema*. It seems probable that inter-racial robbing has also resulted in interchange of disease, with rapidly fatal effects to the respective new hosts.

Humble bees infected with both *Nosema apis* and *N. bombi* have been found near bee hives, and hive bees suffering from a similar mixed infection have also been found from time to time.

One species of humble bee is able to contract infection from another species. This has been shown experimentally by feeding one species of *Bombus* on honey removed from the nest of a different species. The said honey contained faecal matter and spores of *N. bombi* when examined, while workers and young queens from the colony showed infection when dissected and examined. By this method spores from *Bombus agrorum* were used to infect *B. latreillelus*. Similarly *B. terrestris* has been infected by spores from *B. agrorum* and *B. hortorum*, and the reverse experiments also have been made.

The morphology and virulence of the *Nosema* seem unaltered whatever species of *Bombus* is employed as host, and thus the conclusion is that one species of *Nosema*, *N. bombi*, is present in all of them. It should be mentioned that while many colonies examined in July and August were free from infection, in others, especially small ones, none was free from parasites. The queens just emerging from hibernation in May also were examined, and were found to be infected; in fact, the percentage of infected queens was slightly higher than that of infected workers. The drones, obtained best in August, were always few in number, and were seldom infected. Larvae were infected in cases where the original pollen mass on which the eggs were laid had been contaminated by the parental excrement. The mode of infection of the larvae seems to be contaminative. Up to the present, hereditary infection has not been proved, though cases may possibly be found when a larger number of queens has been examined.

VIII. THE ECONOMIC IMPORTANCE OF *NOSEMA BOMBI*

While the economic importance of *Nosema apis*, fatal to hive bees, is very obvious, that of *N. bombi* is less apparent at first. Nevertheless, the parasite may be the cause of serious financial losses should its ravages extend. It is well known that for the pollination and subsequent fertilisation of the red clover, an insect with a long proboscis is necessary, and that this function is performed by humble bees. The setting of the seed of red clover is essential for the production of the next season's crop, but where humble bees are lacking the seed production falls enormously. There is the example of the earlier colonists of New Zealand, who found it necessary to import humble bees in order to save the valuable source of fodder found in the red clover, while usually, in Australia, red clover cannot be cultivated as there are no humble bees there. The lack of humble bees from epizootics such as that due to *N. bombi* has, then, the potentialities of causing direct serious loss of clover and hay, while indirectly there would be a lessening of the natural sources of fertilising the soil, since lack of clover-roots involves necessarily the loss of their symbiotic nitrifying bacteria.

Again, epizootics among humble bees affect many plants from which medicinal substances are obtained, pollination of such plants being brought about mainly by humble bees. The monkshood, *Aconitum napellus*, provides aconite; species of the larkspur, *Delphinium*, yield the alkaloid delphinine; the horse-chestnut yields aesculin, tannin, leucine and a saponaceous substance, and the blossoms of these plants, among others, are visited by humble bees, which, in many cases, are the only insects of use for the purpose of 'setting the seeds.'

The fact that *Nosema bombi* can pass to hive bees with fatal effects has already been mentioned. Weak hives suffer not only from robbing by other hive bees, but also from the attacks of humble bees, who display great patience and determination in effecting an entry. Voiding of excrement commonly occurs during the scrimmage that accompanies the ejection of a humble bee from a hive, and the contamination of hive bees, hive, pollen, etc., is brought about, greatly to the detriment of the hive bees, who rapidly succumb should they ingest spores of *Nosema bombi*. Thus further loss may arise to man from the infection existing among humble bees spreading to hive bees and so cutting off profits from the production of honey and wax.

Should humble bees visit a hive infected with *Nosema apis*, they, like the honey bees, fall victims to the parasite and die of the disease. *Nosema apis* in humble bees appears to undergo no change in morphology in its new host, but its virulence seems intensified. Humble bees may play a very small part in spreading *Nosema apis* from hive to hive.

IX. SUGGESTED PREVENTIVE MEASURES

The control of disease among wild animals is almost impossible, and the difficulty is increased when the parasitised host is a flying insect. When hive bees are infected with *Nosema apis* they are under practically domesticated conditions, their hives are accessible, and seriously infected colonies can be destroyed. The humble bee, producing its colonies in holes in banks, bases of hollow trees, in open fields, and in cavities such as abandoned runs of field-mice, presents a far more difficult problem when diseased. However, when a colony is badly infected, the members often leave the nest, creep or tumble about in the vicinity for some hours, and then become moribund, remaining in this condition for some time. The cold of night usually puts an end to their sufferings. When humble bees are found crawling or lying about dead, they should be collected and burned. Sometimes forty to fifty have been found as described near the foot of the trunk of horse-chestnuts in blossom. When examined microscopically, the presence of *Nosema bombi* was obvious in dying or recently dead bees, though, as with *N. apis*, all stages but the spores perish rapidly after the death of their host. If numbers of *Bombus agrorum* are found dead in a field, it would be well fo try to find the nest, which, with its contents, should be destroyed by fire. If the nest be chloroformed or burnt out with tar or paraffin, as for wasps, in the evening, the greater number of the colony will probably be destroyed with it. If it is destroyed in broad daylight, the population will chiefly be scattered and new centres of disease may be initiated.

It is worth while burning even isolated dead humble bees when they are found. Even if they have died natural deaths, the time is not wasted, and should microsporidiosis be present good is done.

The thoughtless destruction of humble bees' nests by children for the sake of the rank honey they contain is common in certain districts. From the point of view of conserving the race of humble bees, and for the benefit of the red clover crop, this practice should be discouraged as far as possible. Many healthy bees and larvae, frequently larvae destined to become queens of new colonies, are destroyed annually by human agency, and this has a direct, though usually unsuspected, bearing on agriculture.

With regard to the predatory habits of humble bees, the bee-keeper can guard against their attacks by reducing the size of the entry to the hive should the humble bees be numerous. Usually a strong colony of bees easily repels the humble bee, but at times they are serious nuisances. A thin wire stretched across the entry horizontally is no obstacle to the honey bee, but is a deterrent to the larger humble bee. Such precautions tend to conserve the humble bee and hive bee alike by preventing, at any rate to some extent, the interchange of maladies between them.

X. SUMMARY

1. *Nosema bombi*, n. sp., is parasitic in the alimentary canal and Malpighian tubules of various species of humble (or bumble) bees, *Bombus agrorum*, *B. hortorum*, *B. latreillelus*, *B. lapidarius*, *B. sylvarum* and *B. terrestris* being affected. It may also pass naturally to the hive bee, *Apis mellifica*, and to *Apis florea*. It is pathogenic to all the above hosts.

2. The morphology of *N. bombi* resembles that of *N. apis* in its general outlines. The planonts are about 2·2 μ in diameter. Meronts vary in size and in shape with the space in which they were formed. They are most abundant in the Malpighian tubules. The spores are oval, and average 5·2 μ long by 3·7 μ broad.

3. The mode of infection is contaminative, by means of infected food and drink. Larvae can become infected from the food soiled by the parental excrement, in which they hatch out.

4. ***Nosema bombi* can** pass from one species of humble bee to another without change of morphology or virulence. When it reaches hive bees, its facies is preserved, but its pathogenic action is accelerated.

5. The economic importance of *Nosema bombi* is due to the fact that the Bombi are essential for the fertilisation of certain plants of agricultural and chemical importance, the red clover being the best known. The dearth of humble bees due to the action of *N. bombi* has resulted in less red clover seed in certain districts. The possibility of the contraction of microsporidiosis of humble bees by hive bees is also not without significance.

6. Preventive measures from the economic standpoint take two main forms: (*a*) Destruction by burning of all dead humble bees found, and of the nests of moribund bees when they are detected. (*b*) Prevention of the thoughtless destruction of healthy nests, more especially by children. The destruction of the diseased and preservation of the healthy humble bees are both in the interests of agriculturists.

REFERENCES

FANTHAM, H. B., and PORTER, A. (1912). Microsporidiosis, a protozoal disease of bees due to *Nosema apis*, and popularly known as 'Isle of Wight' disease. Annals Trop. Med. and Parasitol., VI, pp. 145-161.

——— ——— (1912). The Morphology and Life-history of *Nosema apis*, and the significance of its various stages in the so-called 'Isle of Wight' disease in bees (Microsporidiosis). Annals Trop. Med. and Parasitol., VI, pp. 163-195. 3 plates.

——— ——— (1912). The Dissemination of *Nosema apis*. Annals Trop. Med. and Parasitol., VI, pp. 197-214.

——— ——— (1912). The Structure and Homology of the Microsporidian spore, as seen in *Nosema apis*. Proc. Camb. Philosoph. Soc., XVI, pp. 580-583.

——— ——— (1913). The pathogenicity of *Nosema apis* to insects other than hive bees. Annals Trop. Med. and Parasitol., VII, pp. 569-579.

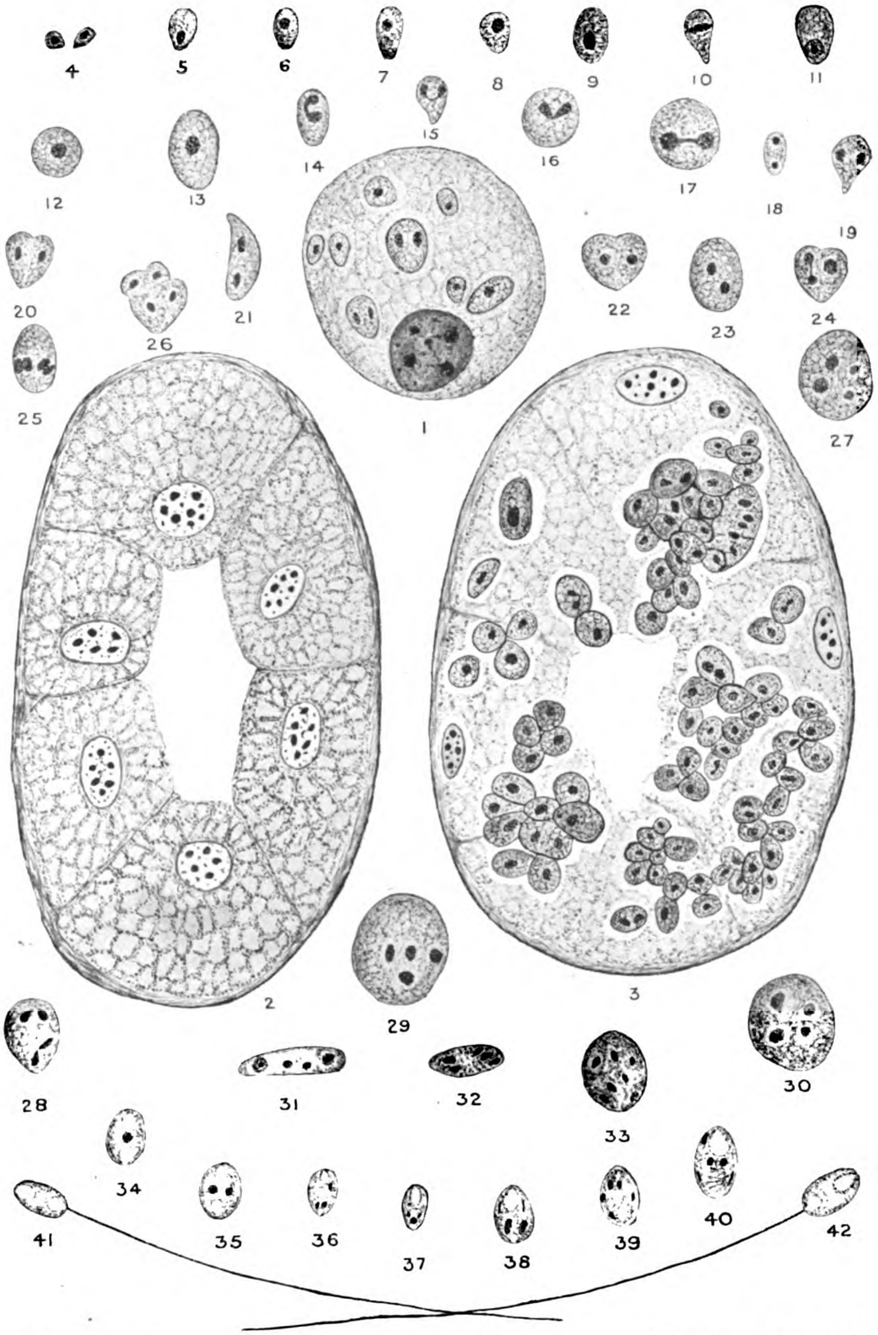
Report on the 'Isle of Wight' Bee disease (Microsporidiosis). Supplement No. 8, Journ. Bd. Agric., May, 1912.

Further Report on the 'Isle of Wight' Bee disease (Microsporidiosis). Supplement No. 10, Journ. Bd. Agric., July, 1913.

EXPLANATION OF PLATE XXX

All figures were outlined with an Abbé-Zeiss camera lucida, and 2 mm. apochromatic or 1/12″ achromatic objective with compensating ocular 8 was used. Magnification is approximately 1300 diameters.

Fig. 1. Cell from alimentary tract of Bombus containing eight *Nosema bombi*, two of which are dividing.
Fig. 2. Normal Malpighian tubule of Bombus, cut slightly obliquely. Excretory products removed during preparation for sectioning.
Fig. 3. Infected Malpighian tubule of Bombus lying parallel to the uninfected one shown in Fig. 2. Nuclei of host cells shrunken and displaced. Many uninucleate meronts, a few binucleate dividing forms and one plasmodial mass of *N. bombi* on the right present. Cytoplasm more feebly staining than in normal tubule.
Fig. 4. Small planonts of *N. bombi.*
Figs. 5-13. Uninucleate meronts or trophozoites of *N. bombi*, showing variation in size and shape.
Fig. 11. Meront with karyosomatic nucleus.
Fig. 12. Spherical meront.
Fig. 13. Larger meront showing alveolar cytoplasm.
Figs. 14, 15. Meronts with nucleus in division, connecting strand distinct.
Fig. 16. Dividing meront, no chromatin strand between the daughter nuclei.
Fig. 17. Meront with dividing nucleus resembling a dumb-bell.
Figs. 18-23. Binucleate meronts showing variations in shape.
Fig. 24. Meront showing division to produce three nuclei.
Fig. 25. Meront with second nuclear division into four in progress.
Figs 26-31. Various appearances of four-nucleate meronts.
Fig. 27. Shows two large and two smaller nuclei in meront.
Fig. 30. Meront with segmentation of cytoplasm in progress.
Fig. 31. Elongate four-nucleate meront.
Fig. 32. Meront with five nuclei.
Fig. 33. Meront with seven nuclei ('plasmodial' form).
Figs. 34-40. Various stages in sporogony.
Fig. 34. Uninucleate spore.
Fig. 35. Binucleate spore, with girdle-like sporoplasm.
Fig. 36. Spore with three nuclei, that of the polar capsule distinct.
Fig. 37. Spore with very minute posterior vacuole and larger anterior vacuole or polar capsule.
Fig. 38. Spore showing two sporoplasmic nuclei and two thread-like sporocyst nuclei.
Fig. 39. Five-nucleate spore, the polar capsule is at the bottom.
Fig. 40. Spore showing full number of nuclei and coiled up polar filament.
Figs. 41, 42. Two spores with polar filaments extruded, after treatment with chemical reagents.



A.P. et H.B.F. del. André, Sleigh & Anglo, Ltd.

NOSEMA BOMBI

STUDIES IN BLACKWATER FEVER

II. A SCHEDULE FOR RECORDING CASES OF BLACKWATER FEVER

BY

J. W. W. STEPHENS, M.D. (CANTAB.)

SIR ALFRED JONES PROFESSOR OF TROPICAL MEDICINE,
THE UNIVERSITY OF LIVERPOOL

(*Received for publication* 28 *October*, 1914)

In analysing the literature of this subject I prepared for my own purposes a schedule for recording various data contained in case reports. I soon found, however, that it was difficult to fill up a schedule satisfactorily owing to various defects in the reports. These may be classified under the following heads:

(1) *Vagueness.* It was not uncommon to find statements like 'the blackwater came on during the night,' 'in the early morning,' 'a few hours after the rigor,' 'shortly after the quinine,' 'urine passed in large quantities,' 'in small amounts,' the 'drug was pushed,' etc. I think it is evident that no very exact meaning can be applied to any of these expressions, so that they are not of much value.

(2) *Diffuseness.* Frequently case reports are verbose, whereas the essential point could be given in a few words; e.g., we have 'at 11 a.m. the temperature began to rise until at 2 p.m. it reached 104°—the maximum during the illness'; i.e., about eighteen words instead of two entries on the temperature chart.

(3) *Omissions.* Not infrequently no mention is made of any particular symptom, e.g., 'jaundice,' 'pain in the loin,' 'hiccough,' 'dyspnoea,' so that it is often quite impossible to say whether a symptom was really absent or not.

(4) *Lack of arrangement.* Again in order to obtain information on a certain point one often had to read through the whole report before it could be ascertained whether or no the point was mentioned.

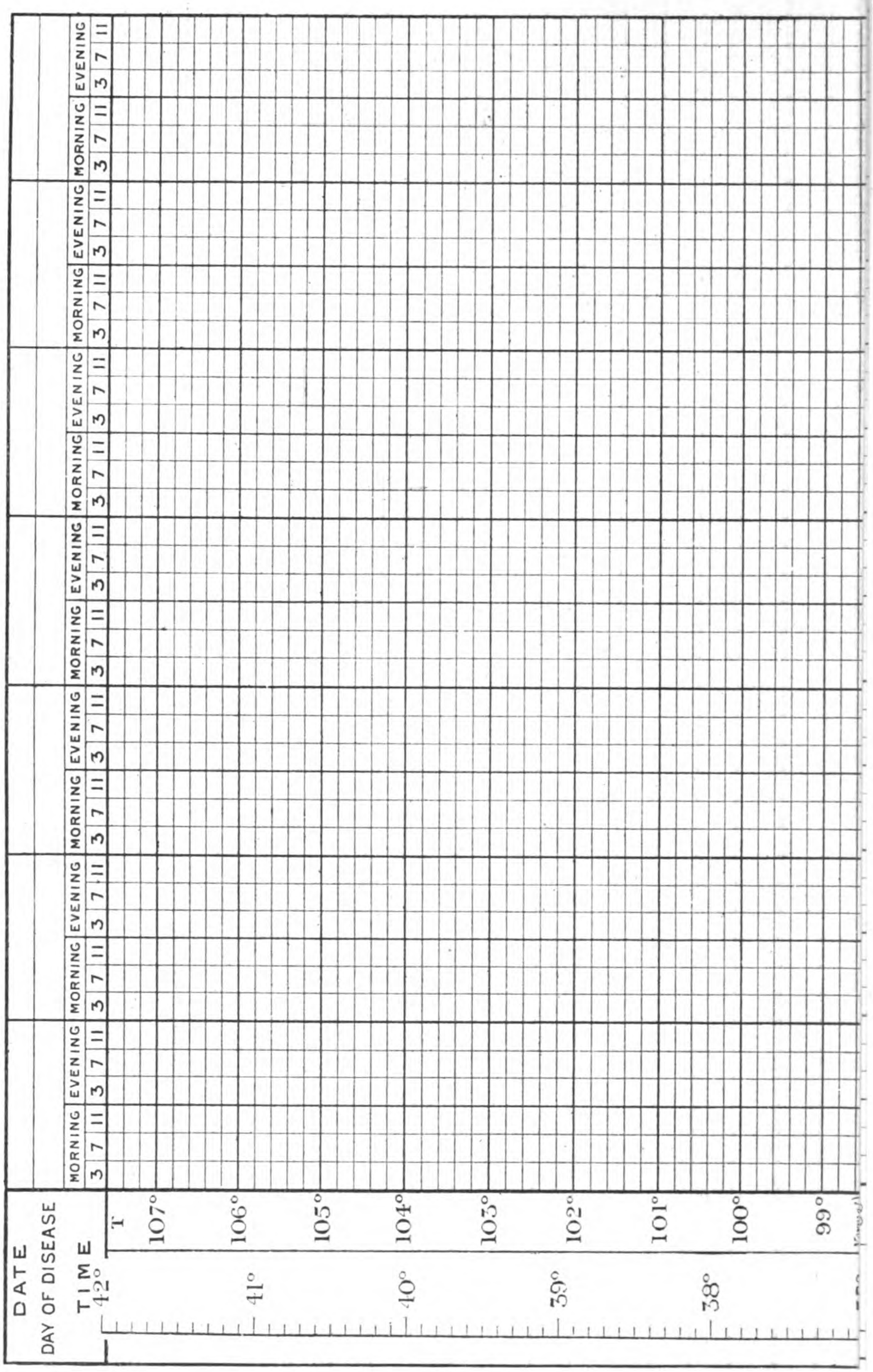
As stated above, I devised this schedule for analysing case reports. I could thus record in a very small space compared to that occupied by the original the essential features of a case. I could, on reference to my schedule, see at a glance the point I wished to ascertain, whether positive or negative.

It seemed to me to be desirable, then, that those recording blackwater cases should do so on some fixed system, and I thought that my original schedule could be easily adapted to that purpose. There were several practical difficulties to overcome, an important one being the limitations enforced on one by considerations of space. One object I have constantly borne in mind has been to burden the recorder with as little writing as possible, and although the schedule may at first sight seem rather formidable, further consideration will show that very little writing has actually to be done, far less than in the ordinary rambling descriptions from which the pith is only extracted with difficulty; and moreover, there is still ample room on the back of the schedule for descriptive reports if anyone feels constrained to write such.

I have carefully considered questions of space and other details, and have had in this respect the great advantage of consultation with medical officers who have themselves written reports of blackwater cases. I hope, therefore, that this schedule may be of use to those recording blackwater cases, and I feel sure that it will both save the recorder much writing and save the person who wishes to analyse such reports a vast amount of trouble.

I have partly filled up one schedule as an example and left the other blank.

BLACKWATER FEVER : *Colony*............................*No*.............



INSTRUCTIONS FOR FILLING THE SCHEDULE

1. Record the times at which all symptoms occur.
2. If a *series* of observations is made at the *same time*, record the time *at the head* of a vertical column.
3. If isolated observations are made at *different* times, record the time (when possible) of each isolated observation in the horizontal space for each symptom, e.g., Hiccough: began 3.15 p.m.
4. Record the symptoms for *different* days in *different* columns.
5. During the acute stage of the disease record *absence* as well as *presence* of symptoms.
6. Avoid as far as possible indefinite expressions. Record, e.g., 2½ hours instead of 'a few hours'; urine 20 ounces instead of a 'fair quantity'; 5 a.m. instead of in the 'early morning.'
7. It is estimated that as a rule one schedule carefully filled up will suffice for each case, but should for example several analyses be made daily of the urine, then several schedules might be required.
8. Any observations of a general character not provided for in the schedule should be written in the blank space above.

Volume VIII January, 1915 No. 4

ANNALS
OF
TROPICAL MEDICINE AND PARASITOLOGY

ISSUED BY

THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Edited by
PROFESSOR J. W. W. STEPHENS, M.D. Cantab., D.P.H.
PROFESSOR R. NEWSTEAD, M.Sc., J.P., F.R.S., A.L.S., F.E.S., Hon. F.R.H.S.
PROFESSOR WARRINGTON YORKE, M.D.
AND
PROFESSOR SIR RONALD ROSS, K.C.B., F.R.S., M.D., F.R.C.S.,
MAJOR I.M.S. (RET.)

Editorial Secretary
DR. H. B. FANTHAM,
School of Tropical Medicine,
The University,
Liverpool.

C. Tinling & Co., Ltd.
Printers to the University Press of Liverpool
53 Victoria Street

THE INCORPORATED LIVERPOOL SCHOOL OF TROPICAL MEDICINE

FOUNDED BY SIR ALFRED LEWIS JONES, K.C.M.G.

(Affiliated with the University of Liverpool)

Hon. President: H.R.H. PRINCESS CHRISTIAN OF SCHLESWIG-HOLSTEIN.
Chairman: MR. F. C. DANSON.
Vice-Chairman: Professor R. CATON.
Hon. Vice-Presidents: THE DUKE OF NORTHUMBERLAND, K.G.
THE EARL OF DERBY, K.G.
EARL CROMER, G.C.B.
VISCOUNT MILNER, G.C.B.
LORD PIRRIE, K.P.
SIR EDWARD MEREWETHER, K.C.V.O.
SIR OWEN PHILIPPS, K.C.M.G.
SIR WILLIAM H. LEVER, BART.
MR. O. HARRISON WILLIAMS

COMMITTEE

Mr. H. J. READ, C.M.G.	*Colonial Office*
Vice-Chancellor SIR ALFRED DALE	*University of Liverpool*
Sir W. B. BOWRING, Bart.	*Council of University of Liverpool*
Professor R. CATON	
Professor W. HERDMAN, F.R.S.	*Senate of University of Liverpool*
Professor J. M. BEATTIE	
Dr. C. J. MACALISTER	*Royal Southern Hospital*
Mr. G. P. NEWBOLT	
Mr. C. BOOTH (Jun.)	*Steamship Owners' Association*
Mr. T. F. HARRISON	
Mr. A. R. MARSHALL	*Shipowners' Association*
Mr. W. ROBERTS	
Mr. J. W. ALSOP	
Mr. G. BROCKLEHURST	
Mr. C. LIVINGSTON	
Mr. J. W. W. DANSON	
Professor E. W. HOPE	
Mr. R. RANKIN	
Professor E. GLYNN	
Mr. E. G. BUCKLEY	
Mr. A. L. REA, *Hon. Treasurer*	
Mr. A. H. MILNE, C.M.G., *Secretary.*	

B 10 Exchange Buildings, Liverpool

Staff, 1915

1. At the University of Liverpool

Professors	JOHN WILLIAM WATSON STEPHENS, M.D., Cantab., D.P.H. *Sir Alfred Jones Professor of Tropical Medicine*
	ROBERT NEWSTEAD, F.R.S., M.Sc., A.L.S., F.E.S., *Dutton Memorial Professor of Entomology*
	MAJOR SIR RONALD ROSS, K.C.B., F.R.S., LL.D., F.R.C.S., D.P.H., M.D., D.Sc. Nobel Laureate 1902. (Indian Medical Service, retired). *Professor of Tropical Sanitation*
	WARRINGTON YORKE, M.D., *Walter Myers Professor in Parasitology*
Lecturers	HAROLD BENJAMIN FANTHAM, D.Sc., B.A., *Lecturer in Parasitology*
	HENRY FRANCIS CARTER, S.E.A.C. DIPL. F.E.S., *Lecturer in Entomology*
	WILLIAM THOMAS PROUT, M.B, C.M.G., *Lecturer on Tropical Sanitation*
	HAROLD WOLFERSTAN THOMAS, M.D., C.M.
	PROF. E. W. HOPE, M.D., D.Sc., *Lecturer on Municipal Sanitation*
Honorary Lecturer	MAJOR JOSEPH FITZGERALD BLOOD, M.D., M.Ch. (Indian Medical Service, retired)
Honorary Statistician	WALTER STOTT

2. At the Yellow Fever Bureau

HARALD SEIDELIN, M.D. (Scientific Secretary).

3. At the Royal Southern Hospital, Liverpool

Physicians	CHARLES JOHN MACALISTER, M.D., F.R.C.P.
	JOHN LLOYD ROBERTS, M.D., M.R.C.P.
Surgeons	D. DOUGLAS CRAWFORD, F.R.C.S.
	ROBERT JONES, F.R.C.S., Ch.M.
	GEORGE PALMERSTON NEWBOLT, M.B., F.R.C.S.
Tropical Pathologist	JOHN WILLIAM WATSON STEPHENS, M.D. Cantab.

4. On Expedition

HAROLD WOLFERSTAN THOMAS, M.D., C.M.

WARRINGTON YORKE, M.D.

B. BLACKLOCK, M.D., D.P.H.

Laboratory

JOHNSTON LABORATORY, UNIVERSITY OF LIVERPOOL

Hospitals :

ROYAL SOUTHERN HOSPITAL AND ROYAL INFIRMARY, LIVERPOOL

Secretary's Office :

B 10, EXCHANGE BUILDINGS, LIVERPOOL

NOTICE

The following courses of instruction will be given by the Liverpool School of Tropical Medicine during 1915:—

Full Course begins 6 January. Advanced Course begins 1 June.
Diploma Examination, 29 March. Certificate Examination, 30 June.
Full Course begins 15 September.
Diploma Examination, 13 December.
These dates are subject to revision.

The full Course of Instruction is open to all qualified medical men, and the Examination to all students who have taken out this full course.

Fee for the full Course of Instruction—Thirteen Guineas.

Fee for the Diploma Examination—Five Guineas.

Fee for the Short Course of Instruction—Four Guineas.

Fee for the use of a School microscope during one term—Ten shillings and sixpence.

For prospectus and further information, application should be made to the Dean of the Medical Faculty, University of Liverpool.

The following have obtained the Diploma in Tropical Medicine of the University of Liverpool:—

Diploma in Tropical Medicine

Date of Diploma	
1904	Augustine, Henry Joshua
1904	Bennett, Arthur King
1904	Bruce, William James
1904	Byrne, John Scott
1904	Clayton, Thomas Morrison
1904	Dalziel, John McEwen
1904	Dee, Peter
1904	Greenidge, Oliver Campbell
1904	Hehir, Patrick
1904	Khan, Saiduzzafor
1904	Laurie, Robert
1904	Maclurkin, Alfred Robert
1904	McConnell, Robert Ernest
1904	Nicholson, James Edward
1904	Philipson, Nicholas
1904	Sharman, Eric Harding
1904	Thomson, Frank Wyville
1904	Walker, George Francis Clegg
1905	Anderson, Catherine Elmslie
1905	Brown, Alexander
1905	Caldwell, Thomas Cathcart
1905	Critien, Attilio
1905	Hooton, Alfred
1905	Hudson, Charles Tilson
1905	Illington, Edmund Moritz
1905	Macfarlane, Robert Maxwell
1905	Maddock, Edward Cecil Gordon
1905	Moore, James Jackson
1905	Nightingale, Samuel Shore
1905	Radcliffe, Percy Alexander Hurst
1905	Young, John Cameron
1906	Adie, Joseph Rosamond
1906	Arnold, Frank Arthur
1906	Bate, John Brabant
1906	Bennetts, Harold Graves
1906	Carter, Robert Markham
1906	Chisholm, James Alexander
1906	Clements, Robert William
1906	Dundas, James
1906	Faichnie, Norman
1906	Jeffreys, Herbert Castelman
1906	Mackenzie, Donald Francis
1906	Pailthorpe, Mary Elizabeth
1906	Palmer, Harold Thornbury
1906	Pearse, Albert
1906	Sampey, Alexander William
1906	Smithson, Arthur Ernest
1906	Taylor, Joseph van Someron
1906	Taylor, William Irwin
1906	Tynan, Edward Joseph

Date of Diploma	
1906	Watson, Cecil Francis
1906	Willcocks, Roger Durant
1906	Williamson, George Alexander
1907	Allan, Alexander Smith
1907	Allwood, James Aldred
1907	Bond, Ashton
1907	Branch, Stanley
1907	Collinson, Walter Julius
1907	Davey, John Bernard
1907	Donaldson, Anson Scott
1907	Fell, Matthew Henry Gregson
1907	Gann, Thomas William Francis
1907	Graham, James Drummond
1907	Hiscock, Robert Carroll
1907	Keane, Joseph Gerald
1907	Kennan, Richard Henry
1907	Kenrick, William Hamilton
1907	Le Fanu, George Ernest Hugh
1907	Mackey, Charles
1907	Maddox, Ralph Henry
1907	McCarthy, John McDonald
1907	Raikes, Cuthbert Taunton
1907	Ryan, Joseph Charles
1907	Vallance, Hugh
1908	Caverhill, Austin Mack
1908	Crawford, Gilbert Stewart
1908	Dalal, Kaikhusroo Rustomji
1908	Dansey-Browning, George
1908	Davidson, James
1908	Dickson, John Rhodes
1908	Dowdall, Arthur Melville
1908	Glover, Henry Joseph
1908	Greaves, Francis Wood
1908	Goodbody, Cecil Maurice
1908	Harrison, James Herbert Hugh
1908	Joshi, Lemuel Lucas
1908	Le Fanu, Cecil Vivian
1908	Luethgen, Carl Wilhelm Ludwig
1908	Mama, Jamshed Byramji
1908	McCay, Frederick William
1908	McLellan, Samuel Wilson
1908	Pearce, Charles Ross
1908	Schoorel, Alexander Frederik
1908	Smith, John Macgregor
1908	Stewart, George Edward
1908	Tate, Gerald William
1908	Whyte, Robert
1909	Abercrombie, Rudolph George
1909	Allin, John Richard Percy
1909	Armstrong, Edward Randolph
1909	Barrow, Harold Percy Waller
1909	Beatty, Guy
1909	Carr-White, Percy
1909	Chevallier, Claude Lionel
1909	Clark, William Scott
1909	Cope, Ricardo
1909	Fleming, William
1909	Hanschell, Hother McCormick
1909	Hayward, William Davey
1909	Henry, Sydney Alexander
1909	Innes, Francis Alexander
1909	Jackson, Arthur Frame
1909	Kaka, Sorabji Manekji
1909	McCabe-Dallas, Alfred Alexander Donald
1909	Meldrum, William Percy
1909	Murphy, John Cullinan
1909	Samuel, Mysore Gnananandaraju
1909	Shroff, Kawasjee Byramjee
1909	Thornely, Michael Harris
1909	Turkhud, Violet Ackroyd
1909	Webb, William Spinks
1909	Yen, Fu-Chun
1910	Brabazon, Edward
1910	Castellino, Louis
1910	Caulcrick, James Akilade
1910	Dowden, Richard
1910	Haigh, William Edwin
1910	Hamilton, Henry Fleming
1910	Hefferman, William St. Michael
1910	Hipwell, Abraham
1910	Homer, Jonathan
1910	Houston, William Mitchell
1910	James, William Robert Wallace
1910	Johnstone, David Patrick
1910	Korke, Vishnu Tatyaji
1910	Macdonald, Angus Graham
1910	Macfie, John Wm. Scott
1910	Manuk, Mack Walter
1910	Murison, Cecil Charles
1910	Nanavati, Kishavlal Balabhai
1910	Nauss, Ralph Welty
1910	Oakley, Philip Douglas
1910	Pratt, Ishmael Charles
1910	Sabastian, Thiruchelvam
1910	Shaw, Hugh Thomas
1910	Sieger, Edward Louis
1910	Sousa, Pascal John de
1910	Souza, Antonio Bernardo de
1910	Waterhouse, John Howard
1910	White, Maurice Forbes
1911	Blacklock, Breadalbane
1911	Brown, Frederick Forrest
1911	Chand, Diwan Jai
1911	Holmes, John Morgan
1911	Ievers, Charles Langley
1911	Iles, Charles Cochrane
1911	Ingram, Alexander
1911	Kirkwood, Thomas
1911	Knowles, Benjamin
1911	Liddle, George Marcus Berkeley
1911	Lomas, Emanuel Kenworthy
1911	Mackarell, William Wright
1911	MacKnight, Dundas Simpson
1911	Mascarenhas, Joseph Victor
1911	Murray, Ronald Roderick
1911	Oluwole, Akidiya Ladapo
1911	Rao, Koka Ahobala
1911	Sinton, John Alexander
1911	Tarapurvalla, Byramji Shavakshah
1911	Taylor, John Archibald
1911	Woods, William Medlicott

Date of Diploma	
1912	Aeria, Joseph Reginald
1912	Anderson, Edmund Litchfield
1912	Borle, James
1912	Bowie, John Tait
1912	Brassey, Laurence Percival
1912	Christie, David
1912	Dillon, Henry de Courcy
1912	Dunn, Lillie Eleanor
1912	Hardwicke, Charles
1912	Jagose, Jamshed Rustomji
1912	Kochhar, Mela Ram
1912	McGusty, Victor William Tighe
1912	Milne, Arthur James
1912	Mitra, Manmatha Nath
1912	Myles, Charles Duncan
1912	Pelly, Huntly Nevins
1912	Prasad, Bindeshwari
1912	Prentice, George
1912	Ross, Frank
1912	Russell, Alexander James Hutchison
1912	Ruthven, Morton Wood
1912	Sandilands, John
1912	Seddon, Harold
1912	Smalley, James
1912	Strickland, Percy Charles Hutchison
1912	Watson, William Russel
1913	Austin, Charles Miller
1913	Banker, Shiavux Sorabji
1913	Becker, Johann Gerhardus
1913	Carrasco, Milton
1913	Clark, James McKillican
1913	Forsyth, Charles
1913	Grahame, Malcolm Claude Russell
1913	Grieve, Kelburne King
1913	Hargreaves, Alfred Ridley
1913	Hepper, Evelyn Charles
1913	Hiranand, Pandit
1913	Jackson, Oswald Egbert
1913	Khaw, Ignatius Oo Kek
1913	MacKelvie, Maxwell
1913	MacKinnon, John MacPhail
1913	Macmillan, Robert James Alan
1913	Mouat-Biggs, Charles Edward Forbes
1913	Noronha, John Carmel
1913	O'Connor, Edward
1913	Olubomi-Beckley, Emanuel
1913	Pestonji, Ardeshir Behramshah
1913	Puttanna, Dodballapur Sivappa
1913	Reford, John Hope
1913	Smith, Edward Arthur
1913	Stewart, Samuel Dudley
1913	Walker, Frederick Dearden
1913	Wilbe, Ernest Edward
1913	Wilson, Hubert Francis
1913	Yin, Ulg Ba
1913	Young, William Alexander
1914	Arculli, Hassan el
1914	Chohan, Noormahomed Kasembha
1914	Connell, Harry Bertram
1914	Gerrard, Herbert Shaw
1914	Gimi, Hirji Dorabji
1914	Gwynne, Joseph Robert
1914	Hodkinson, Samuel Paterson
1914	Jackson, Arthur Ivan
1914	Kaushash, Ram Chander
1914	Kelsall, Charles
1914	Luanco y Cuenca, Maximino
1914	Misbah, Abdul-Ghani Naguib
1914	Naidu, Bangalore Pasupulati Balakrishna
1914	Rowe, John Joseph Stephen
1914	Roy, Raghu Nath
1914	Shiveshwarkar, Ramchandra Vishnu
1914	Sur, Sachindra Nath
1914	Talati, Dadabhai Cursedji
1914	Wilkinson, Arthur Geden
1914	Wright, Ernest Jenner

EDITORIAL NOTICE

By order of the Committee of the Incorporated Liverpool School of Tropical Medicine, the series of the Reports of the School, which had been issued since 1899, were followed, from January 1, 1907, by the Annals of Tropical Medicine and Parasitology, of which this is the fourth number of the eighth volume.

Altogether twenty-one Memoirs, besides other works, were published by the School since 1899, and of these ten, containing 519 quarto or octavo pages and 95 plates and figures, were published during the two years 1904 and 1905.

The Annals are issued by the Committee of the School, and will contain all such matter as was formerly printed in the Reports – that is to say, accounts of the various expeditions of the School and of the scientific work done in its laboratories at the University of Liverpool and at Runcorn. In addition, however, to School work, original articles from outside on any subject connected with Tropical Medicine and Hygiene, and Parasitology may be published if found suitable (see notice on back of cover); so that, in all probability, not less than four numbers of the Annals will be issued annually. Each number will be brought out when material sufficient for it has been accumulated.

BLACKWATER FEVER IN FORMOSA

BY

J. HATORI

SANITARY INSPECTOR, GOVERNMENT OF FORMOSA

(*Received for publication* 26 *October*, 1914)

WITH MAP

INTRODUCTION

Since the Island of Formosa came under the rule of Japan the Japanese settlers have been affected by certain tropical diseases unknown in their motherland, of which blackwater fever is the most dreaded. The actual number of blackwater fever cases and deaths therefrom, being included in that of malaria, is not shown in Government statistics. As to blackwater fever among garrison troops, stationed at different localities of the Island, scientific inquiries have been conducted by the Army surgeons, Prof. Kawashima (Taichu), the late Dr. Shimizu (Hosan), Drs. Tagami (Giran), Mayeshima, Tsuzuki (Taichu), and others. In the Civil Administration Department, Drs. Yoshisawa, the late Kawada, Ugai (Hosan), the late Kabachi, Momma (Taito), Nakagawa, Fujibayashi (Karenko), Ujihara (Tainan), Directors of Civil Hospitals in these respective malarious districts, have enthusiastically investigated and reported on the cases they have treated. In 1911, the Government Committee, appointed to investigate the infectious and endemic diseases of Formosa, prepared forms for the record of blackwater fever, and distributed them to Civil Hospitals and Kois,* and collected a number of reports on cases treated. In this paper I propose to give some facts concerning the disease in this island, omitting pathological, clinical and therapeutical considerations.

---

* Koi is an official practitioner stationed at a particular locality for medical practice.

The places mentioned will be found indicated on the map at the end of this paper.

DISTRIBUTION OF MALARIA AND BLACKWATER FEVER

With the exception of some of the principal cities, malaria exists as an endemic disease throughout the island, varying in its local endemicity and annual prevalence. In some localities, the simple tertian parasite predominates, and in others the principal parasite is the malignant tertian, while the quartan is rather rarely found, with the exception of Yensuiko, where quartan is not so uncommon as in the other localities. For instance, the distribution of parasite species in certain localities is as follows:—

TABLE I. Distribution of the malarial parasites in Formosa.

Locality		No. of parasite carriers	PARASITE SPECIES (per 100)			Author
			Tertian	Quartan	Malignant	
N.—	Hokuto	162	55·0	0	45·0	Hatori (1910)
W.	Chikutoki ...	488	62·1	2·8	35·1	Mori (1911)
	Inrin	716	60·2	17·3	22·2	K. Nakajima (1911)
	Yensuiko ...	1,793	33·5	27·5	39·0	M. Nakajima (1912-3)
S.	Ako	1,080	46·8	16·1	37·1	Kojima (1911-2)
	Hosan	1,658	50·0	13·9	36·1	Kojima (1912)
E.	Karenko ...	2,625	21·75	4·72	71·28	Hatori (1911-2)
	Yoshino	549	44·82	0·36	54·82	Hatori (1911-2)
	Bokusekikaku ...	345	22·9	8·10	69·0	Hatori (1911)

(N., W., S. and E. denote Northern, Western, Southern and Eastern Formosa respectively).

TABLE IA*

Locality	Number Examined	Positive	Parasite Rate °/oo
Inrin	11,703	716	61·19
Yensuiko	43,277	1,793	41·43
Ako	35,445	1,080	30·47
Hosan	56,512	1,658	29·34
Karenko	78,401	2,625	33·48
Yoshino	20,689	549	26·54
Bokusekikaku	16,128	345	21·39

As a rule, malaria rages among new-comers such as the labourers engaged in the building of railways, and among the employees of sugar and camphor factories, and also among Japanese immigrants, &c. The deaths caused by malaria are, however, gradually decreasing year by year. The following table shows the malarial deaths and rates per 1,000 of the total population.

TABLE II. Malaria death-rate (per 1,000) in Formosa.

Year	Number of deaths			Death-rate per 1,000
	Japanese	Formosans and Foreigners	Total	
1906	295	10,267	10,562	3·44
1907	244	11,441	11,685	3·77
1908	183	11,519	11,702	3·75
1909	197	10,136	10,333	3·26
1910	180	8,924	9,104	2·82
1911	178	7,771	7,949	2·41
1912	195	6,714	6,909	2·06

---

* These figures should be compared with those given in Table II, 'The Anti-malarial Campaign in Formosa.' Hatori. *Annals of Trop. Med. & Parasit.* 1914. Vol. VIII, p. 541.

The malarial death rates in different prefectures and among different races vary greatly, as shown in the following figures for the year 1912.

TABLE III. Malaria death-rate (per 1,000) among the different races in Formosa, 1912

	Prefecture	MALARIAL DEATH-RATE PER 1,000 INHABITANTS			Total
		Japanese	Formosans	Foreigners	
N.	Taihoku	0·46	0·68	1·38	0·68
	Giran	2·23	2·04	6·06	2·13
	Toyen	0·44	0·61	...	0·61
	Shintiku	0·84	0·93	...	0·93
	Average	0·6	0·91	1·35	0·9
W.	Taichu	2·51	1·43	...	1·47
	Nanto	2·81	2·04	...	2·06
	Kagi	2·08	3·15	2·33	3·12
	Average	2·35	2·26	1·04	2·26
S.	Tainah	1·21	1·77	3·8	1·76
	Ako	4·3	6·42	...	6·35
	Average	1·93	3·23	3·24	3·18
E.	Taito	7·31	6·08	20·00	6·15
	Karenko	7·01	7·42	4·05	7·34
	Average	7·08	6·61	8·64	6·73
Pesc.—Hoko		...	0·16	...	0·15
	Total average	1·59	2·07	1·84	2·06

The following table shows the cases of malarial fever (including blackwater) treated in 11 Civil Hospitals in 1912.

TABLE IIIA. Malaria deaths for 1912.

Prefecture		Japanese	Formosans	Foreign	Total
N	Taihoku	22	309	15	346
	Giran	8	286	1	295
	Toyen	1	132	0	133
	Shintiku	4	292	0	296
	Total	35	1,019	16	1,070
W	Taichu	28	812	0	840
	Nanto	9	237	0	246
	Kagi	27	1,681	2	1,710
	Total	64	2,730	2	2,796
S	Tainah	27	948	12	987
	Ako	29	1,571	0	1,600
	Total	56	2,519	12	2,587
E	Taito	9	200	2	211
	Karenko	31	204	1	236
	Total	40	404	3	447
	Hoko (Pescadores)	0	9	0	9
	Total	195	6,681	33	6,909

TABLE IV. Cases of Malaria (including blackwater) among the patients treated in 11 Civil Hospitals in 1912.

Civil Hospital		Number of cases of malaria and blackwater fever	Cases per 1,000 of total patients
N.	Tihoku	106	3·5
	Kirun	156	30·2
	Giran	592	65·7
	Shinchiku	164	27·6
W.	Taichu	673	109·9
	Kagi	1,262	110·8
S.	Tainan	583	37·7
	Ako	1,187	168·4
E.	Taito	1,319	241·4
	Karenko	744	147·7
Pesc.	—Hoko	156	23·0

From the above tables, it may easily be understood that malaria severely infests the eastern and southern parts of the Island, where blackwater fever likewise occurs most frequently (*vide* the accompanying map). Dr. Yoshisawa observed 1·43 % blackwater cases among the total number of malaria cases treated at the Hosan Civil Hospital (1906-7), Dr. Nakagawa 1·51 % at Karenko Civil Hospital (1910-11), and his successor, Dr. Fujibayashi, 2·22 % (1912).

The following numbers of cases of blackwater fever were reported to the Committee by the Civil Hospitals and some Kois in 1912; the localities where these cases occurred are marked in the map.

TABLE V. Blackwater fever cases in Formosa during 1910.

	Reported by	Number of cases	
N.	Taihoku Civil Hospital	1	8
	Kirun Civil Hospital	0	
	Giran Civil Hospital	5	
	Shinchiku Civil Hospital	0	
	2 Kois	2	
	20 Kois	0	
W.	Taichu Civil Hospital...	6	12
	Kagi Civil Hospital	3	
	2 Kois	3	
	12 Kois	0	
S.	Tainan Civil Hospital...	4	41
	Ako (Hosan) Civil Hospital*	33	
	2 Kois	4	
	15 Kois	0	
E.	Taito Civil Hospital	4	23
	Karenko Civil Hospital*	13	
	1 Koi	6	
Pcs.	Hoko Civil Hospital	0	0
	4 Kois	0	
	Total	84	

(These figures are not the actual total number, there may be numbers of unreported cases.)

In localities where anti-malarial work is carried out, all parasite-carriers and fever cases microscopically diagnosed are submitted gratis to a strict systematic treatment with quinine preparations, namely, pastilles of quinine hydrochloride or *pulvis* euchinin or *pill*, 'Esanopheles,' and at the beginning of or during the course of the treatment, blackwater is occasionally met with. As shown by the following table, the ratio of blackwater fever cases to the total number of cases treated for malaria differs greatly according to the locality.

---

* For populations of corresponding districts see Table XIV.

TABLE VI. Cases of malaria and blackwater fever during 1911 and 1912.

Part and locality			1911						1912					
			Total number of cases treated for malaria		Number of blackwater fever cases		‰		Total number of cases treated for malaria		Number of blackwater fever cases		‰	
N.	Hokuto ...	...	92		0		0		182		0		0	
	Shokirrun	...	156		0		0		148		0		0	
	Kimpori...	...	154		0		0		124		0		0	
	Ginaryo	...	254		0		0		471		0		0	
W.	Chikutoki	J.	443	1,207	2	2	4·51	1·66	298	802	0	0	0	0
		F.	764		0		0		504		0		0	
	Yensuiko	...	...		...		...		735		0		0	
S.	Hosan ...	J..	206	1,200	0	0	0	0	140	1,111	0	0	0	0
		F.	994		0		0		971		0		0	
	Ako ...	J	691	1,518	0	0	0	0	485	954	0	0	0	0
		F	827		0		0		469		0		0	
	Banshiho	...	...		...		...		61	165	3	4	49·18	24·24
									104		1		9·62	
E.	Karenko	J.	1,290	2,690	10	11	7·75	4·09	1,285	2,401	16	18	12·45	7·50
		F.	1,400		1		0·71		1,116		2		1·79	
	Yoshino ...	J.	578		3		5·19		1,210		1		0·83	
	Rigyobi ...	...	...		...		...		52	96	2	2	38·46	20·83
									44		0		0	
	Bokusekikaku	J.	384	915	1	1	2·60	1·09	239	532	2	2	8·37	3·76
		F.	531		0		0		293		0		0	
	Shinnun	...	...		...		...		127		0		0	

(J. = Japanese. F. = Formosans and Chinese.)

RACIAL RELATIONSHIP

According to the latest records the total population of Formosa in 1912 amounted to 3,343,943 (excluding the wild aboriginal tribes inhabiting the central mountains, whose number exceeds 120,000), of which 122,792 are Japanese. The great majority of the inhabitants of the island consists of 3,213,221 Formosans (whose ancestors were settlers from South China), besides non-savage aborigines who live in the plain. The remaining 7,930, classified as foreigners, are chiefly composed of Chinese labourers from Canton and Fukiang Provinces. Of these races, the Japanese have the greatest susceptibility to blackwater, the Chinese taking second place, while the Formosans and aborigines have the least. The latter especially are very rarely attacked, only two cases of blackwater have hitherto been known among them. From this fact, it can be realised that want of acclimatisation, especially want of malaria immunity, is one of the important factors in the pathogenesis of blackwater fever. The racial relationship to blackwater can be seen from the following table.

TABLE VII. Blackwater fever among the different races in Formosa.

Locality	BLACKWATER CASES AMONG			Total cases	Author
	Japanese	Formosans	Chinese		
Taito Civil Hosp. ...	6	0	0	6	Kabachi (1905)
———	38	1	0	39	The committee (1910)
Hosan and Ako C. H.	59	2	0	61	Yoshisawa (1910-11)
Karenko C. H. ...	32	2	5*	39	Nakagawa (1910-1)
Karenko‡	26	1†	2*	29	Hatori (1911-2)
Total	161	6	7	174	

(* Chinese labourers. † Two-year old boy of Formosan father and aboriginal mother.)

‡ The population of Karenko town in 1913 consisted of 2,415 Japanese, 1874 Formosans and 47 Foreigners (i.e. Chinese).

Blackwater fever is seldom found to attack father and son or daughter (observed at Taichu and Rigyobi) or brother (observed at Karenko); but it sometimes attacks two persons who live together in the same house.

RELATIONSHIP TO SEX AND AGE

Blackwater fever usually attacks adults from 20 to 40 years of age, while it is less frequently observed in infants, youths, old men and females. This difference is chiefly due to the fact that adult men are more frequently exposed to malaria, and so to blackwater.

TABLE VIII. Age and sex distribution of blackwater fever.

Author and date	Total number of cases	AGE							SEX	
		1-10	11-20	21-30	31-40	41-50	51-60	61—	Male	Female
The committee (1910)	39	0	2	15	17	5	0	0	33	6
Yoshisawa (1910-1)	61	3	6	24	24	4	0	0	53	8
Nakagawa (1910-1)	39	1	2	18	15	2	1†	0	31	8
Hatori (1911-2) Karenko	29	2*	4	15	7	1	0	0	22	7
Total	168	6	14	72	63	12	1	0	139	29
%	...	3·57	8·33	42·86	37·50	7·14	0·60	0·0	82·74	17·26

(* Youngest case is 2 years' old native half-cast boy. † 59 years' old Japanese.)

TABLE VIIIA. Age distribution of the population of Karenko (October, 1912).

1-10	11-20	21-30	31-40	41-50	51-60	61—	Total
549	476	981	747	377	105	33	3,268

THE SEASONAL PREVALENCE

Though blackwater fever occurs throughout the year in Formosa, it is less prevalent in the months of March, August and September, as the following table shows.

TABLE IX. The distribution by months of blackwater fever cases in Formosa.

Locality and date	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total	Author
Taichu (1900) ...	9	14	5	8	5	13	2	0	1	1	12	5	75	Tsuzuki
Tainan (1900) ...	1	0	2	1	6	7	5	3	0	0	0	0	25	Tsuzuki
Hosan & Ako (1910-1)	8	4	2	8	5	10	7	2	1	4	4	6	61	Yoshisawa
Karenko (1910-1) ...	4	2	0	2	8	3	4	5	2	6	2	1	39	Nakagawa
Karenko (1911-2) ...	1	3	3	5	3	1	3	0	0	3	3	4	29	Hatori
Total	23	23	12	24	27	34	21	10	4	14	21	16	229	

TABLE IXA. Distribution by months of malaria cases in Karenko (Hatori)

(1911) April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	(1912) Jan.	Feb.	Mar.
368	154	179	212	157	169	322	279	292	241	174	143
150	189	194	230	158	143	179	347	314	(1913) 201	127	85

MALARIAL PARASITES

It is a well-known fact that at the time of the onset of blackwater, malarial parasites totally disappear from, or conspicuously decrease in number in, the peripheral blood of the patient. Dr. Nakagawa (Karenko) observed parasites in 85 % of cases examined previous to the onset, 14 cases with malignant tertian, two with simple tertian, and one with quartan parasites, while in cases examined one day or two days after the onset only, 12·8 % were positive.

During my anti-malarial work at Karenko, in 26 cases of parasite carriers (and fever cases) blackwater occurred on the day of, or some days after quinine, which had been administered immediately or some days after the finding of parasites.

TABLE X. Malarial parasites in blackwater fever.

Number of positive cases	Positive on the day of onset	NUMBER OF DAYS BEFORE ONSET WHEN LAST FOUND POSITIVE								
		1 day	2 days	3 days	4 days	5 days	8 days	11 days	13 days	60 days
25	2	7	3	3	3	1	1	2	2	1

In only one fever case did I fail to find parasites on the day before and on the day of onset of blackwater, though the case was undoubtedly one of malarial fever. In my cases the findings regarding parasites were positive in 96·3 % and negative in 3·7 % prior to the onset of blackwater. As to the species of the parasites detected by me in my 26 cases, the malignant tertian was found in 25 cases, and the simple tertian parasite in one case. Mixed infection with parasites of different species should occur in malarious districts, and it is interesting to note in this connection that Dr. Nakagawa observed a case of blackwater fever in which he found malignant tertian parasites prior to the onset and simple tertian parasites after subsidence of the blackwater, so he believed this to be a case of mixed infection.

LENGTH OF RESIDENCE IN MALARIOUS LOCALITY

Length of residence in malarious districts makes some people predisposed to blackwater, as the result of chronic malaria there acquired. Almost all Formosan authors (Drs. Shimizu, Tagami, Yoshisawa, and others) agree that the length of residence from the time of arrival in a malarious district until the first attack, is generally over seven months. There are, however, some cases which occur after shorter periods of residence, for example, a case was observed by Dr. Nakagawa after one month's stay in a malarious district and another by Dr. Ugai after about two months. The majority of blackwater cases that occur in towns such as Karenko are imported from malarious districts or occur in patients who have already been seriously predisposed to the disease before their arrival. The disease chiefly attacks such labourers as coolies, carpenters, peasants, &c., and policemen, who, overworked, poorly nourished and with imperfect medical aid, live in or visit malarious localities. It is, moreover, a well-known fact that these

				DAY OF ONSET			Recovery or death	Remarks
4	3	2	1	Parasites	Drugs	Onset occurred		
……	fever	……	S.T.	……	……	p.m.	R.	
……	……	……	……	……	Q4 × 1			
……	fever	M.T.	……	……	……	p.m.	R.	
……	……	Q2 × 4	Q2 × 4	……	Q2 × 4			
……	……	M.T.	……	……	……	p.m.	R.	
……	……	Q2 × 4	Q2 × 4	……	Q2 × 4			
……	fev	……	M.T.	……	……	p.m.	R.	
……	……	……	Q2 × 4	……	Q2 × 4			
……	……	……	……	……	……	p.m.	R.	Second attack 6 months later. Recovery.
……	……	……	Q2 × 4	……	Q8 × 1			
……	……	……	……	……	……	p.m.	R.	
……	……	Q8 × 1	Q8 × 1	……	Q8 × 1			
……	……	……	……	……	……	p.m.	R.	
E2 × 3	E2 × 3	E2 × 3	E2 × 3	……	E6 × 1			
……	……	……	……	……	……	p.m.	R.	
……	……	Q8 × 1	Q8 × 1	……	Q8 × 1			
……	……	……	……	……	……	p.m.	R.	There was a second attack with Recovery.
……	……	……	……	……	Q2 × 3			
fever	……	……	neg.	neg.	……	p.m.	R.	Later another attack; death.
……	……	……	Q2 × 4	……	Q2 × 4			
……	……	……	……	M.T.	……	p.m.	R.	
……	……	……	……	……	Q2 × 2			
……	M.T.	……	……	……	……	p.m.	R.	
……	……	Q2 × 4	Q2 × 4	……	……			
……	……	……	M.T.	……	……	p.m.	R.	Second attack some months later Death.
……	……	……	……	……	Q2 × 5			
M.T.	……	……	……	……	……	p.m.	D.	
Q2 × 5(?)	Q2 × 5(?)	Q2 × 5(?)	Q2 × 5(?)	……	Q6 × 4			
……	……	……	M.T.	……	……	m.	R.	
……	……	……	Q2 × 4	……	Q4 × 1			
……	……	……	……	M.T.	……	p.m.	R.	
……	……	……	……	……	Q2 × 4			
……	M.T.	……	……	……	……	p.m.	R.	
……	Q2 × 4	Q4 × 4	Q4 × 4	……	Q4 × 1			
……	……	……	……	……	……	p.m.	D.	Brother of K.H., case 26.
……	……	……	Q10 × 1	……	Q10 × 1			
M.T.	……	……	……	……	……	a.m.	R.	
……	……	Q8 × 1	Q8 × 1	……	……			
……	……	……	M.T.	……	……	p.m.	D.	
……	……	……	Q2 × 4	……	Q2 × 4			
……	……	……	……	……	……	p.m.	R.	2 days later second attack, following 1 grain Euchinine.
……	……	E2/3	……	……	E2/3			
M.T.	……	……	……	……	……	p.m.	R.	
E3 × 4	E3 × 4	E3 × 4	E3 × 4	……	E6 × 1			
……	……	……	……	……	……	a.m.	R.	
E3 × 4	E3 × 4	E3 × 4	E3 × 4	……	……			
……	M.T.	……	……	……	……	m.	R.	
……	E3 × 4	E3 × 4	E6 × 2	……	E3 × 4			
……	fever	M.T.	……	……	……	p.m.	R.	
……	……	E3 × 4	Q2 × 1	……	E2 × 3			
……	……	……	M.T.	……	……	p.m.	R.	Brother of R.H., case 18.
……	……	……	Q10 × 1	……	Q10 × 1			
……	……	……	……	……	……	p.m.	R.	
E3 × 4	E3 × 4	E3 × 4	E3 × 4	……	Q4 × 2			
……	……	……	……	……	……	p.m	R.	
E2 × 3	……	……	……	……	E7 × 1			
……	……	……	M.T.	……	……	p.m.	R.	
……	……	……	……	……	E3 × 4			

panese.

ormosans.

Chinese.

for 59th-18th day are omitted to economise space.

species in cases 5 and 28 reported by Dr. Ibusuki, the Koi.

individuals take quinine in undetermined quantities and at irregular intervals. So that, as a result of chronic malaria due to repeated relapses and re-infection, the haemolytic disposition is induced in them. On the contrary, Dr. Nakagawa recorded a case of blackwater in a Chinese woman who, according to her statement, was suffering from her first infection with malaria.

QUININE AS IMMEDIATE CAUSE OF BLACKWATER FEVER

According to general observations, blackwater occurs in the great majority of cases after quinine administration for the treatment or prevention of malaria. The length of time between the taking of quinine and the onset of blackwater varies in different cases from about half an hour to many hours or a day or more.

Although the onset usually comes quite abruptly after the first daily dose or doses, there are some cases in which blackwater only develops later, several days or more, after quininisation. For instance, according to my own observations on 26 cases at Karenko, where the anti-malarial quininisation of parasite carriers (and fever cases) was continued for two months after Nocht's method, in 6 cases blackwater appeared after the first dose or first day of taking quinine; in 8 cases after 2 days' quininisation; in 3 cases after 3 days, in 2 after 4 days; in 2 after 5 days, and in each of three cases after 6, 8 and 10 days respectively, in 2 cases after 11 days, and in the longest one after about 2 months (these latter cases with interruptions of 3 to 5 days).

The accompanying table (Table XI, opposite) gives some details of the above observations. Dr. Nakagawa observed at the Karenko Civil Hospital 2 cases in which the blackwater occurred 2 or 3 days after admission into the hospital, 3 cases after 4 days, and 1 case after 5 days.

Many authors (Drs. Yoshisawa, Nakagawa, Hatori and others) have observed the occurrence of blackwater in individuals who, being afraid of contracting blackwater or out of sheer neglect (as in the case of the Chinese), did not take any quinine at all for malaria. In some cases the patients declared that the taking of a quack medicine brought on the attack of blackwater. Thymol-haemoglobinuria, though quite different from malarial blackwater, has been observed by Dr. Yoshida in a certain number of Formosans during a course of treatment for ankylostomiasis; two of the cases ended fatally.

RECURRENCE OF BLACKWATER FEVER

It appears from the history of those who live in malaria-stricken localities, and who have had blackwater fever once and are attacked again after a shorter or longer interval of time, as if the haemolytic disposition persisted in them. Dr. Yoshisawa observed at Ako the case of a patient who had five attacks during 10 years' residence in South Formosa and who died from the sixth attack, 2 other cases of third attacks and 5 cases of second attacks. Of 29 cases under my observation at Karenko, 1 case was a third attack, 5 cases were second attacks (2 of them fatal), while the other 23 cases were first attacks.

MORTALITY FROM BLACKWATER FEVER IN FORMOSA

The mortality of blackwater fever, according to various authors in Formosa, ranges from 10 to 25 %. Among garrison troops, the rate is lower than among civilians, owing to the age of the patients, &c.

TABLE XII. Case mortality in Formosa.

(*a*).—Among garrison troops

Date	Troops at	Number of B.W. cases	Number of deaths	Case mortality %	Author
1897	Giran	19	3	15·79	Tagami
1896-7	Taichu	30	3	10·0	Kawashima
1900	Taichu	75	11	14·67	Tsuzuki
1900	Tainan	25	3	12·00	Tsuzuki
1895-6	Hosan	46	5	10·87	Shimizu

(*b*).—Among civilians

Date	Locality	Number of B.W. cases	Number of deaths	Case mortality %	Author
1910	...	39	8	20·51	The committee
1910-1	Hosan and Ako ...	61	11	18·03	Yoshisawa
1910-1	Karenko	39	10	25·64	Nakagawa
1911-2	Karenko	29	3	10·34	Hatori

Blackwater fever is more fatal to those who have already had a primary attack, as the following table shows.

TABLE XIII. Mortality during first and subsequent attacks of blackwater fever.

Author	FIRST ATTACKS			RECURRENCES			UNCLASSIFIED		
	Number of cases	Recovery	Deaths	Number of cases	Recovery	Deaths	Number of cases	Recovery	Deaths
The committee	27	22	5	7	5	2	5	4	1
Nakagawa	35	27	8	4	2	2	0	0	0
Hatori	23	22	1	6	4	2	0	0	0
Total	85	71	14	17	11	6	5	4	1
%	...	83·53	16·47	...	64·71	35·29	...	80·0	20·0

The annual blackwater death rate per 1,000 inhabitants in certain localities where anti-malarial measures are in progress is as follows: —

TABLE XIV. Blackwater death-rate in malaria-protected localities, 1911-1912

Locality	1911			1912		
	Average population	Black-water deaths	°/oo	Average population	Black-water deaths	°/oo
Hokuto	1,500	0	0	1,670	0	0
Chikutoki	1,870	0	0	1,625	0	0
Ako	5,400	2	0·37	5,997	2	0·33
Hosan	...	...	...	6,270	3	0·48
Karenko	2,836	3	1·06	3,537	2	0·57
Yoshino	...	...	...	1,160	1	0·86
Bokusekikaku	1,001	0	0	1,115	0	0

In conclusion, blackwater fever in Formosa generally is diminishing in amount and becoming milder in nature. This decrease is well-marked in the most renowned fever districts, such as Bokusekikaku, Ako, Taito, &c. Recent statistics of garrison troops show a great decrease or entire disappearance of the disease among the soldiers.

TABLE XV. Blackwater fever among garrison troops,* 1908-1913.

Garrison Hospital	BLACKWATER FEVER CASES TREATED DURING						Total
	1908	1909	1910	1911	1912	1913	
Taihoku, Taikokan, Kirun, Giran, Taichu, Polisha, Hoko, Koshun	0	0	0	0	0	0	0
Tainan	3	1	4	1	2	3	14
Hosan	4	3	4	1	1	...	13
Karenko	9	8	20	3	2	3	45
Bokusekikaku	0	0	0	0	0	1	1
Taito	2	0	1	0	0	0	3
Total	18	12	29	5	5	7	76

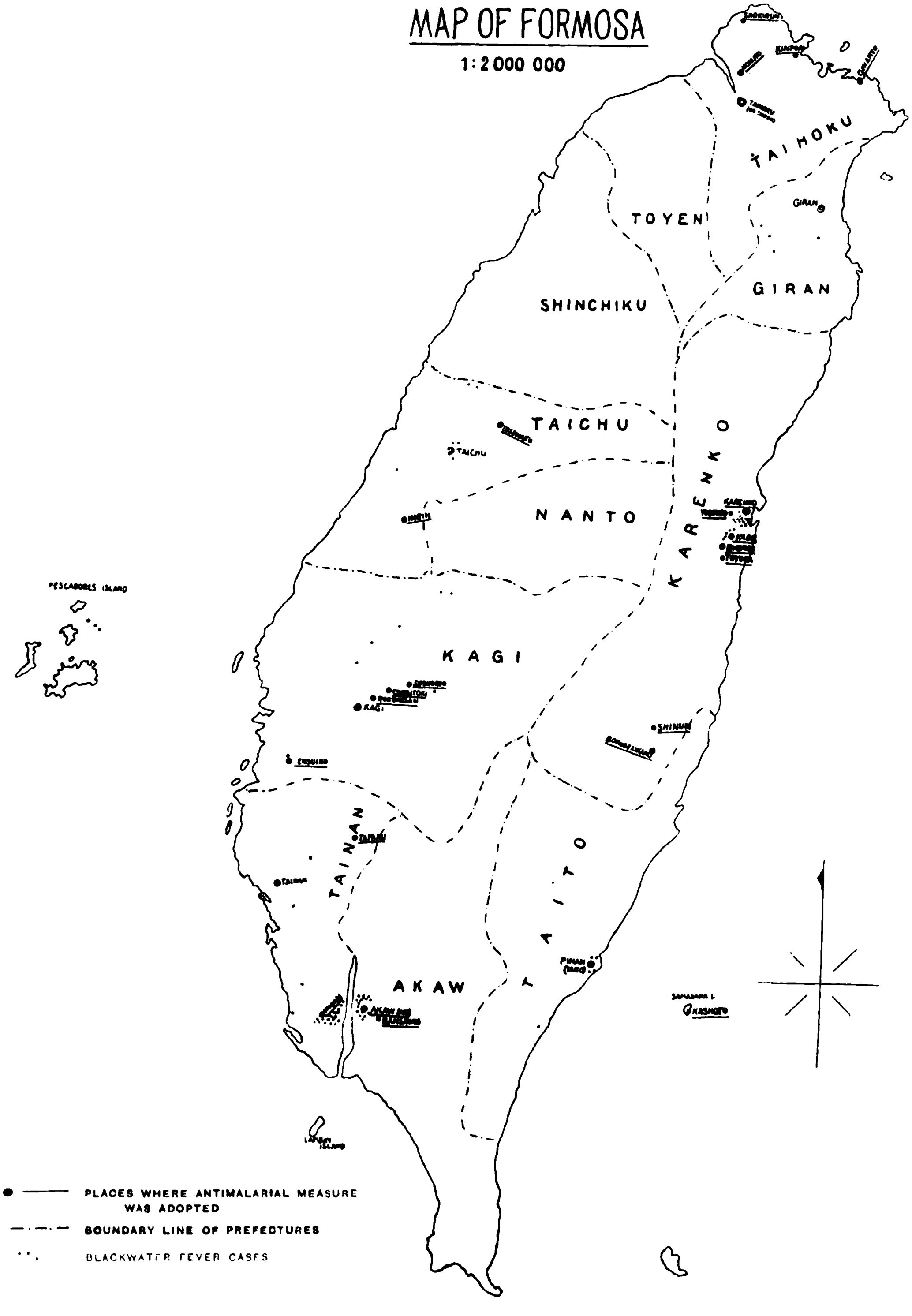
This result is chiefly due to the anti-mosquito measures adopted by the garrison troops and to improved sanitary conditions of the environs. As the result of anti-malarial measures throughout the island, it may be hoped that blackwater fever will diminish *pari passu* with malaria.

---

* The strength of the troops in various districts is unknown.

MAP OF FORMOSA

1:2 000 000



SPLENOMEGALY: BEING A REPORT ON A HUNDRED AND FOUR CASES MET WITH IN SOUTH CHINA

BY

G. DUNCAN WHYTE, M.D. (EDIN.), D.T.M. & H. (CAMB.)

(*Received for publication* 29 *October*, 1914)

THREE CHARTS

SPLENOMEGALY

In examining a patient suffering from malarial cachexia it is an easy matter to determine whether the spleen is or is not enlarged; it may be a much more difficult matter to discover the etiology of an obvious splenomegaly.

An endeavour has been made to elucidate the cause of the splenomegalies treated year by year in the hospital at Swatow, and over one hundred cases have been examined with this purpose. Most of the cases met with give a very chronic history (see Chart II), and all of them show very great enlargement, so they are embraced within the category 'Chronic and very great enlargement of the spleen,' which, according to a recent text-book on Medical Diagnosis,[1] may be due to one of the following causes:

Splenomedullary leukaemia,
Lymphatic leukaemia,
Mixed leukaemia,
Chronic malaria,
Kala-azar,
Splenomegalic polycythaemia,
Splenomegalic cirrhosis,*
Splenic anaemia,
Pseudo-leukaemia infantum.

Two additions to this list must be made, viz., 'Histoplasmosis,' described by Dr. Darling in the Panama Canal Zone,[2] and

---

* Splenomegalic cirrhosis is not mentioned in the indices either of Osler's or Dieulafoy's text-books of medicine. According to French (1), it 'occurs in children and young people, and is characterised by enormous enlargement of the spleen, slight enlargement of the liver, anaemia without leucocytosis, haematemesis, clubbing of the fingers, jaundice, and stunted growth.

Egyptian Splenomegaly, described by Drs. Day and Ferguson.[3] One might think, too, of congenital syphilis as a cause of splenic enlargement, but in that condition the degree of enlargement is rarely so great as in the cases under consideration.

One may anticipate the detailed study of the blood conditions so far as to say that in no case did the red cells exceed 5,000,000 nor the white cells 6,500 per cubic millimetre. These facts definitely exclude both polycythaemia and the leukaemic conditions,* so that in seeking to find a cause for the Swatow splenomegalies one is faced with the possibilities of malaria, kala-azar, and splenic anaemia, as well as the two more recently described conditions to which reference has been made.

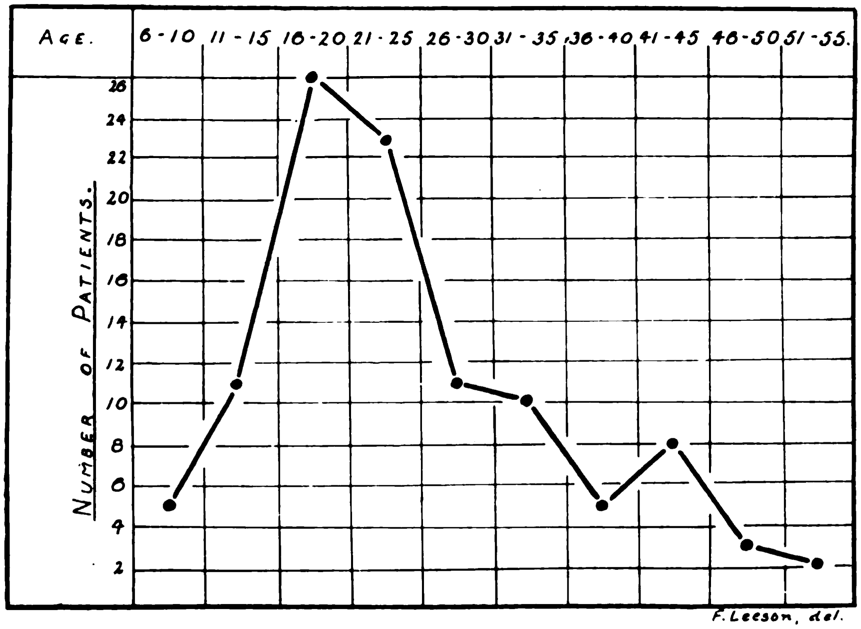
It may be stated at once that it is impossible to differentiate any one of the Swatow cases from Banti's disease, which is defined in Osler's text-book of medicine as: 'A primary disease of the spleen of unknown origin, characterised by progressive enlargement, attacks of anaemia, a tendency to haemorrhage, and in some cases a secondary cirrhosis of the liver with jaundice and ascites.' The paper will show how completely the cases described meet the clinical side of the description of Banti's disease. 'It is a disease of young and middle life, the majority of cases occurring before the fortieth year.'[12] Chart I shows that 90 per cent. of the cases were under forty. 'The spleen is uniform, smooth and painless.'[12] The spleen was always smooth, and only rarely had there been any pain in the region of the spleen. The enlargement of the spleen 'may exist for years without any other symptom.'[12] The average duration of splenic enlargement before the patient came to hospital was seven years.

And so it would be possible to go through the description of splenic anaemia in this or almost any recent text-book noting that the characteristic symptoms of Banti's disease are also met with in every case now under consideration. Even the existence of malaria is not sufficient to justify a diagnosis of malarial splenomegaly as opposed to Banti's disease, for Osler notes 'in a few of my cases malaria has been present,' and in a recent text-book on blood diseases we are told that 'a variety of conditions, such as malaria, has preceded it' (i.e., splenic anaemia).[4]

---

* Two cases of splenomedullary leucocythaemia were met with, but the features presented by both differed so greatly from those of the 104 cases hereinafter described that a further consideration of them would only tend to confuse the question under consideration.

Let us consider, then, the history of the illness and the various symptoms met with in the Swatow cases, noting, in each instance, whether the facts elucidated have any bearing on the differentiation of these cases from malarial cachexia, Banti's disease, and kala-azar.*



F. Leeson, del.

Chart I.—Showing the age period during which the illness began (in 104 cases).

History: *Age at which the illness began.* This is shown in Chart I, from which it will be seen that practically half the cases were between sixteen and twenty-five years of age when they first became aware of a 'lump' in the position of the spleen. In kala-azar 50 per cent. of Rogers's cases were below twenty. It has already been pointed out that what Professor Osler says about the age at which splenic anaemia commences is in fullest accord with what was found in Swatow.

---

* In dealing with the symptoms of the first two conditions reference will be made to text-books of general medicine, and to those dealing especially with diseases of the blood, as well as to books on tropical medicine. In regard to Kala-azar, many writers have quoted from, but few have been able to add anything to, the wonderful accumulation of relevant facts which is to be found in Leonard Rogers' 'Fevers in the Tropics.' The description he gives of Kala-azar will be accepted as the standard.

Is it a family disease? 'There is marked family tendency and heredity'[5] in Banti's disease, which often 'has occurred in more than one member of the same family.'[4] In kala-azar, 80 per cent. of the patients have at least one relative affected. In the Swatow cases 40 per cent. told of relatives who were suffering from enlarged spleen, etc., but it was noted that this percentage varied with the district from which the patient came: e.g., eighteen patients were distributed over three districts and only three of these (i.e., 17 per cent.) had relatives affected, whereas of thirty-eight patients who came from one single district, in as many as twenty-four cases (i.e., 63 per cent.) at least one relative was affected. This suggests that if there are many cases of splenomegaly in a district it is to be expected that some of these will be amongst the relatives of the patients coming to hospital, whereas if only few cases exist in that region a smaller number of patients will have relatives who are affected. Perhaps there is little more than this in the 'family' incidence of kala-azar.

The duration of illness up to the time the patient came to hospital varied between a few months and (in one case only) thirty years. In 28 per cent. of the cases the illness had lasted for ten or more years. The course of Banti's disease is said to be 'extraordinarily chronic,'[12] and a case is recorded[5] in which, although splenomegaly had been discovered thirteen years previously, the patient still enjoyed fairly good health and was able to pursue his occupation daily. In malarial cachexia the majority of cases recover under treatment. In kala-azar, on the other hand, patients may be expected to die in about three years.

SYMPTOMS

Coming, then, to a consideration of the symptoms from which the patient had suffered, we take up, in the first place:—

Fever. 88 per cent. of the patients remembered having had attacks of fever, and in two-thirds of these cases the form of infection was either simple or double tertian, the remainder being cases of quartan fever. It may cause surprise that as many as 12 per cent. failed to give a history of fever, but Sir Patrick Manson says that 'in highly malarious countries it is not unusual to see typical examples [of malarial cachexia] in which fever has never been a feature, or has been of so slight a character as not to have seriously attracted attention, or had occurred in childhood and been forgotten.'[6] Banti's disease is 'generally' characterised by 'pyrexial attacks,'[1] and a history of fever and rigors is usually obtainable in kala-azar.

Oedema or Ascites had been present, or was found when the patients came to hospital, in 62 per cent. of the cases. One or other is a usual symptom in kala-azar, ascites is present in some cases of Banti's disease,[12 4] oedema of the ankles is frequently present in malarial cachexia.[12 7]

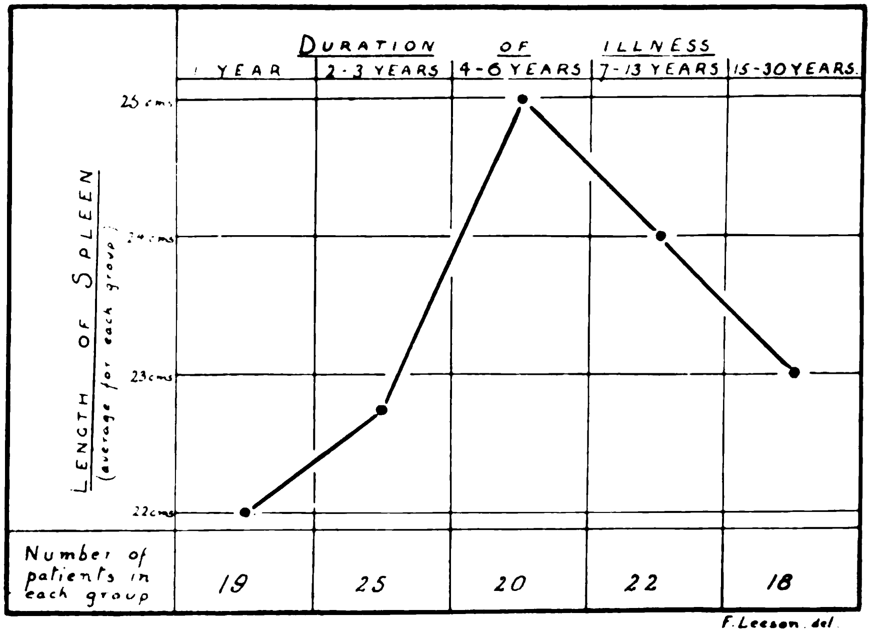
Haemorrhage—usually from the bowel or the nose—was recorded in 61 per cent. of the cases, and this also corresponds with the picture in Banti's disease, in which a tendency to haemorrhage is recognised by all,[4 5 7 12] though Osler notes that in this condition the form of bleeding is 'usually haematemesis'—which has hardly ever been mentioned by the Swatow patients. In malarial cachexia 'haemorrhages of various kinds are apt to occur; epistaxis . . . . haematemesis . . . . melaena.'[6] Haemorrhages are also of frequent occurrence in kala-azar.

Diarrhoea was recorded in 50 per cent. of the cases. In Banti's disease 'gastro-intestinal disturbance with sickness and diarrhoea'[5 7] are often met with. In kala-azar, 30 per cent. of the cases give a history of diarrhoea, but in malarial cachexia the number is not so large as a rule.

Physical signs. These being the symptoms of which a history can be obtained what do we find on making an examination of the patient? Even a most casual glance at one of these cases generally shows that a distinct degree of anaemia is present—but this point will be dealt with in detail when the condition of the blood is considered. Another point that often impressed one on looking at the patients was their emaciated appearance; but on weighing and measuring them it was found that only one-fourth weighed much less (i.e., more than 10 per cent. less) than the calculated standard weights for their respective heights.*[8] I can find no mention of emaciation in the description of Banti's disease: Rogers states that it is not *always* present in kala-azar, but adds that 'in malarial cachexia the wasting is much less marked than in . . . kala-azar.' When the abdomen is inspected the splenic enlargement is the most striking feature. The smallest spleens measured 13 × 11 cm. and 14 × 9 cm., while the largest were 53 × 23 cm. and 41 × 24 cm. The average length was $23\frac{1}{2}$ cm. It is interesting to note that the spleen does not continue to increase in size during the whole course of the patient's illness, but that after a certain period a gradual diminution in size can be made out. Thus, while one patient with a small spleen (13 × 11 cm.) had only been ill for three months, the very smallest spleen was met with in a man who had been ill for twenty years. By classifying the patients according to the duration of their illness one can obtain five classes each containing about twenty cases (Chart II). A comparison of the average length of spleen in each of these classes shows that while in each successive class from one to six years' duration the average length of the spleen increases, yet after that period it becomes less again, evidence, doubtless, of a cirrhotic process succeeding the hypertrophy of the earlier years.

---

* The fact that the appearance of emaciation is not confirmed by weighing the patients may be explained, in some cases, by the obvious oedema or ascites, and in others by the probable occurrence of an (undetected) oedema of the viscera and deep tissues.



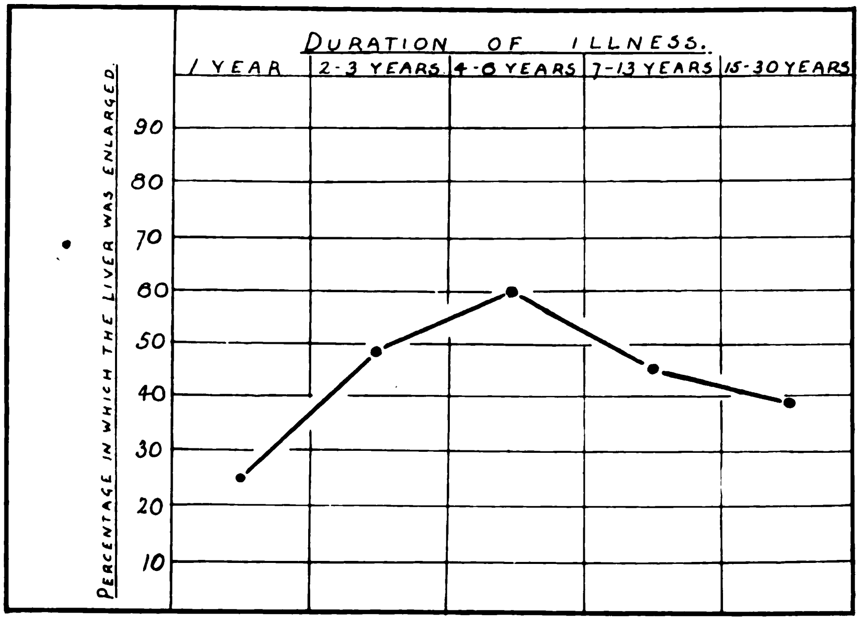
F. Leeson del.

CHART II.—Showing the relation between the duration of the illness and the size of the spleen.

[In this Chart and the following one, the last column of ages is headed 15-30 years by the author. Perhaps it should have been 14-30 years, but as the author is in China, it is not possible to inquire of him directly.—ED.]

In malaria cachexia 'the spleen is gradually reduced in size,'[3] but no such change has been described in Banti's disease. In kala-azar, as 70 to 98 per cent. of the patients die before the fourth year, it is not unnatural that a diminution in the size of the spleen has not yet been recorded.

The Liver. The state of the liver usually varied with the duration of the disease. In only one quarter of the Swatow patients examined during the first two years of their illness was the liver enlarged, whereas in those who had been ill for a longer time, hepatic enlargement was present in as many as half the cases. On the other hand, it was found that of the patients who had been ill for fourteen years or more, only one-third showed an enlarged liver—as shown in Chart III below.



F Leeson del.

Chart III.—Showing the relation between the duration of the illness and enlargement of the liver.

It will be noted that clinically the pathological processes in the spleen and in the liver run fairly parallel to one another, first of all gradual enlargement, reaching its acme between the fourth and sixth years, and then gradual contraction.

That the liver is affected in Banti's disease is acknowledged by various authorities,[2, 4, 7] while Leonard Rogers states that 'the liver may also be enlarged in malarial cachexia, but does not so often reach the extreme degree of extension—as far as the level of the navel—as it does in kala-azar.' Such a degree of hepatic enlargement was never met with in the Swatow cases.

BLOOD EXAMINATION

Having dealt thus with the principal physical signs found on examining the patient, we are in a position to discuss the results of examining the blood.

The haemoglobin values[*] are given in the following table (Table I), which shows that in more than three-fourths of the cases the haemoglobin was between 50 and 70 per cent.[†]

TABLE I.—The percentage of haemoglobin.

Haemoglobin value	Number of cases
40 %	3 cases
50 %	20 cases
60 %	19 cases
70 %	32 cases
80 %	17 cases

In Banti's disease,[7] kala-azar, and malarial cachexia,[10] the haemoglobin is reduced.

The *erythrocytes* varied in number from 2½ to 4 millions per cubic millimetre in more than half the cases. The majority of the remaining cases showed between four and five millions, though a few (11 per cent.) had less than two and a half millions. In Banti's disease there seems to be a consensus of opinion[4 5] that from two and a half to three million red cells is the number usually met with, though 'it may fall as low as two million.'[12] In kala-azar, too, there is a considerable reduction in the number of red cells, although at first the degree of anaemia is not so great as that met with in malarial cachexia; 54 per cent. of Rogers's cases of kala-azar gave from two and a half million to four million per c.mm.[10]

The *colour index* varied in different cases from 0·6 to 1·3, but in two-thirds of the cases it did not exceed 0·9, which agrees with the condition met with in Banti's disease, where 'the rule is a colour index below unity.'[5] In malarial cachexia an index of less than one is also to be expected,[10] [12] whereas in kala-azar the colour index is normal.[10]

---

[*] The percentage of haemoglobin was estimated with Talquist's apparatus, which is the simplest: if the tests are carefully carried out the results are sufficiently accurate for comparative purposes.

[†] In considering the degree of anaemia shown by these figures it should be borne in mind that both Captain McCay (9) and Leonard Rogers (loc. cit) have come to the conclusion that the 100 per cent. standard of haemoglobin is rarely if ever reached by the vegetarian natives of India. Similarly one has failed to find a Chinaman with more than 95 per cent. haemoglobin, the average of recent observations on healthy natives of Swatow being only 90 per cent.

Whether *normoblasts* were or were not present seemed to depend mainly on the degree of reduction of the total number of red cells. Thus nucleated red cells were found in one-half the cases whose total red count was less than two and a half million, and in only 6 per cent. of the cases with more than four million cells. In the intermediate group normoblasts were found in 30 per cent. of the cases. Normoblasts may be found in a majority of cases of Banti's disease,[4] but their presence in kala-azar is rare.

Poikilocytes were noted in 27 per cent. of the cases examined. It is on record that they are sometimes present in splenic anaemia, though only rarely in kala-azar. I have found no record as to their presence or absence in cases of malarial cachexia.

Leucocytes. The following table shows the result of an enumeration of the leucocytes in each case:

Table II.—Number of leucocytes per cubic millimetre
(In 102 patients at Swatow, and in 76 cases of Kala-azar)

Number of cells	Percentage of cases at each figure	
	Swatow patients	Rogers's patients
500—1000	4 %	42 % below 1000
1001—1500	10 %	30 % 1001—2000
1501—2000	26 %	
2001—2500	16 %	22 % 2001—3000
2501—3000	10 %	
3001—3500	9 %	2½ % 3001—4000
3501—4000	8 %	
4001—4500	2 %	
4501—5000	6 %	2½ % above 4000
5001—5500	3 %	—
5501—6000	3 %	—
6001—6500	3 %	—

Two-thirds of the cases have less than 3,000 white cells. This fact shows how much these different diseases—splenic anaemia, malarial cachexia, and kala-azar—have in common, for not only is leucopenia present in all of them, but it is one of the most important diagnostic signs. 'The association of an enlarged spleen with leucopenia is almost alone sufficiently diagnostic' of splenic anaemia (Fleming), but it is equally characteristic of the other conditions.

We come next to a point on which Leonard Rogers has laid some stress in differentiating malaria and kala-azar. 'The ratio of the white to the red corpuscles will generally allow of their [i.e., these two diseases] being readily distinguished, for in malaria they are reduced in about equal proportions, while in uncomplicated kala-azar the white are reduced at least twice as much as the red, so that the proportion of white to red is less than 1 to 1,500, and commonly much lower.'

The following table (Table III) shows the ratio between the white and the red cells in the Swatow splenomegalies, and also in Leonard Rogers's kala-azar cases:

TABLE III.—Ratio between white and red cells

One leucocyte to	Percentage of cases at each figure	
	Swatow patients	Rogers' patients
750 (or fewer) red cells	14·7 %	4 %
751—1000	13·7 %	9·2 %
1001—1500	26·4 %	17·1 %
1501—2000	20·6 %	18·4 %
2001—3000	20·6%	15·8 %
3001—4000	2 %	19·7 %[1]
4001 (and upwards)	2 %	15·8 %

A glance at the table reveals the fact that the Swatow cases do not correspond exactly with either of the groups described by Rogers. On the one hand they do not show the normal ratio of white to red cells—which he affirms is present in malaria—and on the other hand more than half of them do not show the ratio of less than 1 to 1,500 which was present in over two-thirds of his kala-azar cases, and is, he affirms, characteristic of this condition. This important point will be referred to again later on.

The results of the differential count of the white cells (based on an examination of 300 cells) is shown in the following tables (Tables IV, V, VI, and VII):

TABLE IV.—Percentage of polymorphonuclear cells met with in the Swatow patients.

Percentage of polynuclears	Number of cases
35·1—40 %	5 cases
40·1—45 %	6 cases
45·1—50 %	11 cases
50·1—55 %	20 cases
55·1—60 %	15 cases
60·1—65 %	17 cases
65·1—70 %	16 cases
70·1—75 %	10 cases
75·1—80 %	1 case

TABLE V.—Number of polymorphonuclear cells per c.mm.

Number of cells	Percentage of cases at each figure	
	Swatow patients	Rogers's patients
0—250	—	12 %
251—500	2 %	32 %
501—1000	18 %	33 %
1001—2000	54 %	20 %
2001—3000	23 %	3 %
3001—4000	4 %	—

TABLE VI.—Percentage of large mononuclear cells.

Percentage of large mononuclears	Percentage of cases at each figure	
	Swatow patients	Rogers' patients
0—8 %	35 %	12 %
8·1—12 %	43 %	19 %
12·1—15 %	13 %	26 %
15·1 % upwards	9 %	43 %

TABLE VII.—Percentage of small mononuclear cells (lymphocytes) met with in Swatow patients.

Percentage of small mononuclears	Number of cases
5·—10 %	1 case
10·1—15 %	4 cases
15·1—20 %	13 cases
20·1—25 %	28 cases
25·1—30 %	16 cases
30·1—35 %	15 cases
35·1—40 %	18 cases
40·1—45 %	8 cases
45·1—50 %	2 cases
50·1—55 %	1 case

How do the facts shown in these tables compare with the blood-counts found in the different diseases we have been considering? In Banti's disease there appears to be 'no constancy in the differential count.'[5] Some authorities find that 'there are no special changes in the differential count,'[12] at any rate before the third stage,[4] but others believe that 'a relative increase of lymphocytes is common with a corresponding relative decrease in the polymorphonuclear neutrophiles.'[5] In malaria and kala-azar, on the other hand, the differential counts are quite definite: 'leucopenia, with a high percentage of lymphocytes, especially the larger forms,' is acknowledged by all authorities to-day* as characteristic of both. Rogers states, further, that the determination of a high percentage of large mononuclear cells 'is of very little value in the diagnosis of malaria' in those tropical countries in which kala-azar exists.

If Tables IV to VII are considered in the light of these quotations, what deductions is one to draw as to the nature of the splenomegalies met with in Swatow? It seems that one is compelled to exclude kala-azar, for although in most of the cases the mononuclear cells are more numerous than usual (Table VI), yet they are certainly not as much increased as in Leonard Rogers's kala-azar cases.† Further, although the polymorphonuclear cells are definitely diminished in number, usually a smaller percentage than normal (Table IV), and always a smaller number per cubic millimetre (Table V), yet there is not so great a reduction as in kala-azar—in which 77 per cent. of the cases have less than 1,000 polymorphonuclear leucocytes per cubic millimetre. This view—that kala-azar is not the cause of the Swatow splenomegalies—is confirmed by a consideration of many of the facts brought out in this paper, especially the ratio of red cells to white cells, and the very different prognosis in these two conditions. It finds further support in Dr. Cochran's report that 'the numerous cases [giving typical kala-azar symptoms] reported from . . . Swatow . . . if interpreted in the light of the negative examinations reported in similar cases in the neighbourhood, would seem not to be kala-azar.'[13] The blood-counts in Dr. Cochran's cases of kala-azar in China are given in Table VIII below.

---

* And yet in 1904 Cabot was able to state that 'In post-malarial anaemia Billings, Thayer, and De Costa have found quite marked leucocytosis.'(11)

† It must be borne in mind that various observers do not agree with Leonard Rogers in attaching such great importance to the blood picture. Thus Dr. Cannata (14), in describing 56 cases of kala-azar (Mediterranean form), reports that in 40 per cent. there was a complete absence of leucopenia, and one-fourth of these showed a certain degree of leucocytosis. Note also the reports from China and South America on the blood-count in Kala-azar (Table VIII).

TABLE VIII.—Showing the results of blood examination in various cases of enlarged spleen.

	Cases of Kala-azar			Other Splenomegalies	
Country … … …	India	South America	North China	South China	Eg
Observer … … …	Rogers	Migone[4]	Cochran	Whyte	Day & F
No. of cases … …	80	1	12	104	4
Erythrocytes … …	2½-4 million	3,900,000	2,304,000	3,600,000	2,65
Leucocytes … …	700—2000	3400	2360	2650	45
Ratio of white to red cells	Less than 1 : 1500	1 : 1147	1 : 1200	1 : 1713	1 : 5
Polymorphonuclears …	—	49 %	47·5 %	56 %	62
Polymorphonuclears per c.mm. …	Less than 2000	1666	1121	1486	[illegible]
Mononuclears, including lymphocytes	60 %—80 %	46 %	50 %	39 %	28

Although many of the symptoms shown by the Swatow patients resemble those of Egyptian splenomegaly—'moderate anaemia with more or less wasting,' 'slight fever of an irregular type,' hepatic cirrhosis and occasionally haematemesis[3]—yet the almost constant occurrence of marked ascites in the Egyptian cases (as compared with its infrequence among the Swatow patients), and the difference between the blood-picture shown by the Egyptian and the Swatow patients (Table VIII above), warrant the belief that the two diseases own a different causation.

On the other hand, there is no positive evidence to show that the Swatow patients differ from the cases which occur in the Canal Zone, and are characterised by 'splenomegaly, irregular pyrexia, leukopenia, anaemia, emaciation, and chronicity.'[2] This condition has been differentiated from kala-azar by the absence of the Leishman-Donovan body, and the presence of a small encapsulated micro-organism which Darling has named the *Histoplasma capsulatum.* Neither of these bodies has been found in the peripheral blood of the Swatow patients, and in no case has either the liver or the spleen been punctured in the search for causal organisms.

Rolleston, in differentiating Banti's disease from chronic splenic anaemia, states that the latter condition presents the following characters: '(i) Chronic splenomegaly, which cannot be correlated with any recognised cause; (ii) absence of enlargement of the lymphatic glands; (iii) chlorotic anaemia, namely, with a low colour index; (iv) absence of leucocytosis, and usually the presence of leucopenia; (v) liability to copious gastro-intestinal haemorrhages from time to time; (vi) the prolonged course without any tendency to spontaneous cure.'[15]

This list shows that it is impossible to be sure that any particular one of the Swatow patients was not a case of splenic anaemia, but as this is a rare condition,* and as no one has yet described the occurrence of splenic anaemia in endemic form, one cannot believe that that is the proper diagnosis for the majority of these cases.

CONCLUSIONS

1. That, in spite of a careful consideration of the symptoms and an examination of the blood, it is impossible to say whether the Swatow patients were suffering from malarial cachexia, histoplasmosis, or some hitherto undescribed condition.

2. That one or more cases of splenic anaemia may have been included in the 104 Swatow splenomegalies, because the clinical differentiation of these two conditions is impossible.†

---

* Only ten cases of splenic anaemia and Banti's disease were examined in the pathological department of the London Hospital during the years 1907 to 1913.

† The difficulty of diagnosing Banti's disease is shown not only in the findings of this paper, but still more prominently in the Transactions of the Royal Society of Medicine, where it is on record that four cases of splenic anaemia were described which some of the more experienced gentlemen in the audience believed to be cases of haemolytic icterus, and not splenic anaemia at all. To discover whether haemolytic icterus was the cause of the Swatow splenomegalies, samples of blood from a selection of these patients were diluted with sodium chloride solutions varying in strength from 0·1 per cent. to 0·9. As none of the cases tested showed undue fragility of the red cells, haemolytic icterus could be definitely excluded.

3. That if the cases which have been described are malarial cachexia, then some of the signs which have been found useful in India in differentiating malarial cachexia from kala-azar—e.g., the ratio of the leucocytes to the haemocytes—do not apply to patients in South China.

4. That 'experience is fallacious and judgment difficult.'

REFERENCES

1. FRENCH, HERBERT. An Index of Differential Diagnosis of Main Symptoms. 1912.

2. DARLING, S. T. Histoplasmosis. Archives of Internal Medicine, September, 1908. The Lesions of Histoplasmosis, a fatal disease of Tropical America. Journal of Experimental Medicine, 1909.

3. DAY, H. B., and FERGUSON, A. R. An account of a form of Splenomegaly with Hepatic Cirrhosis, endemic in Egypt. Annals Trop. Med. and Parasit., Vol. III, No. 3, 1909.

4. GULLAND, G. L., and GOODALL, A. The Blood. 1912.

5. BUCHANAN, R. J. M. The Blood in Health and Disease. 1909.

6. MANSON, Sir PATRICK. Tropical Diseases. 1908.

7. FLEMING, R. A. Short Practice of Medicine. 1902 (2nd edition).

8. WHYTE, G. D. Notes on the Height and Weight of the Hoklo People of the Kwangtung Province, South China. Journal Royal Anthrop. Inst., Vol. XLI, 1911.

9. McCAY, Captain D. Scientific Memoirs by officers of the medical and sanitary departments of the Government of India. No. 34, 1908.

10. CASTELLANI, A., and CHALMERS, A. J. Manual of Tropical Medicine. 1913 (2nd edition).

11. CABOT, R. C. A guide to the clinical examination of the blood. 1904 (5th edition).

12. OSLER, Sir WILLIAM. Principles and Practice of Medicine. 1912.

13. COCHRAN, S. Some observations on Kala-azar in China. Journal London School of Tropical Medicine, 1913, Vol. II, Part III, p. 179.

14. CANNATA, S. La Pediatria. 1912, No. 8. (Abstract in Trop. Dis. Bull., I, p. 363).

15. ROLLESTON, H. D. The Practitioner. April, 1914.

16. MIGONE, L. E. Un cas de Kala-azar à Asuncion (Paraguay). Bull. Soc. Path. Exot., February, 1913.

17. Trans. Roy. Soc. Med. (Clinical Section), February 13, 1914.

NOTES ON YAWS IN CEYLON, WITH SPECIAL REFERENCE TO ITS DISTRIBUTION IN THAT ISLAND AND ITS TERTIARY MANIFESTATION

BY

PHILIP H. BAHR, M.A., M.D. (CANTAB.), M.R.C.P. (LOND.),
D.T.M. & H. (CAMB.)
(FROM THE LONDON SCHOOL OF TROPICAL MEDICINE)

(*Received for publication* 19 *December*, 1914)

PLATE XXXI

Yaws is a common disease amongst the poorer agricultural labourers in Ceylon, where it is known colloquially as '*Parangi.*' There are certain aspects of the disease as it occurs in Ceylon which are as yet little understood, and with regard to which I have been enabled to make some observations perhaps worthy of publication.

EPIDEMIOLOGY

Though widespread in certain clearly defined areas, yaws appears to have a definite topographical distribution in Ceylon, a significant fact, which I suspect has not so far received the attention it deserves. In the Northern and Eastern Provinces which constitute the dry belt of Ceylon, yaws is so prevalent that almost every villager in the jungles near the townships of Anurhadhapura, Polonnuruwa, Jaffna and Trincomalee is conspicuously marked with cicatricial scars, so characteristic of the past ravages of this disease. Similarly amongst the 'goiyas' or paddy-field labourers in the hot, damp, malaria-stricken villages of the Southern Province the same rule holds good. On the other hand, amongst the Sinhalese and Tamil estate labourers in the comparatively cool up-country planting districts the disease appears to be unknown, though syphilis in all its forms is prevalent enough. Indeed, it has been my experience, as a result of my enquiries, that cases of yaws in which the original infection was contracted at an elevation of 800 or more feet above sea-level, are extremely rare.

There are difficulties, I admit, in the differential diagnosis of certain stages of syphilis from yaws on clinical signs alone, yet I was unable, as far as my enquiry would permit, to verify the existence of a venereal infection amongst the jungle villagers who, as I have already stated, are nearly all the subjects of yaws. This observation would certainly seem to suggest that there is developed in man a reciprocal immunity between these two obviously closely allied diseases, a fact which thus constitutes an analogy, considered in an epidemiological sense, with the condition observed by myself (1912) in Fiji and Tonga, island groups in which yaws is almost universal, but syphilis quite unknown.

These preliminary and somewhat rough observations received additional support from others during a tour I made of the principal hospitals in the Island. In the Badulla Hospital, at an elevation of 2,000 feet, I did not see a single case of yaws, though one ward was set apart and devoted to the treatment of syphilitics alone; in another hospital, some sixty-nine miles distant and at a considerably lower elevation—400 feet—almost every patient bore conspicuous yaws scars; in Batticaloa again, a town at sea-level on the East Coast of Ceylon, still another forty miles distant, yaws is so prevalent as to necessitate the reservation in the hospital of two special wards for its treatment. From this it may be legitimately inferred that the actual temperature of the atmosphere is the one climatic factor governing this somewhat restricted distribution. The immediate environment, such as housing, food, the nature of the clothing, may all be disregarded as a means of disseminating the virus, as they are not materially different in natives of the same social status, whether inhabitants of the low-lying jungles or of higher elevations.

Provided with these data it may be permissible to speculate on the circumstances capable of determining a topographical distribution of this character. It has occurred to me that the distribution of yaws, a disease in which, as far as is known, the causative spirochaete is probably at one time of its existence distributed throughout the blood-stream as well as in the framboesiform eruptions, might be limited by the same factors as control other well-known protozoal diseases, as, for instance, relapsing fever, and that a blood-sucking insect—a biting fly or a mosquito—whose range is also definitely limited by climatic factors or the character of the vegetation already referred to, might be concerned in the conveyance of the specific spirochaete from man to man. One thing is certain, that no evidence can be adduced indicating that yaws is a venereal infection, and the theory of inoculation by direct contact does not, I venture to think, explain its peculiar regional distribution in Ceylon. One might almost suggest that the two closely related spirochaetes—*Spirochaeta pallida* and *Sp. pertenuis* —may, in respect of their mode of transmission, occupy a position parallel to that of other morphologically identical protozoa, the *Trypanosoma equiperdum* ('Mal du coït') and *Trypanosoma gambiense*.

Although the house-fly (*Musca domestica*) has on experimental grounds been incriminated as a conveyer of the yaws spirochaete in Ceylon by Castellani (1907), I submit that in this island, at least, on epidemiological grounds, there is little to support this supposition. Firstly, because this insect being unprovided with piercing mouth organs, and from the nature of its habits is unable to inoculate the germ on an unbroken skin. Secondly, because this fly is far more abundant in the elevated planting districts where the conditions appear to be more favourable for its propagation, and where yaws appears to be unknown, than in the low country where the disease is common.

SOME CLINICAL FORMS OF YAWS

I wish to draw attention to some of the clinical manifestations of this disease.

As in syphilis, so in yaws, the clinical manifestations are protean in character. The primary lesion of yaws is generally asserted to be a small hard papule; such I have seen situated generally on the buttocks, but I have been unable to decide whether any of these small lesions were in reality of this nature. The characteristic framboesiform secondary rash was not often observed, and then only in children or in young adults in the endemic zones. It is, however, the tertiary lesions and sequelae (or, at least, those which I believe to be tertiary lesions) which appear to be little known and insufficiently described, to which I specially wish to draw attention. Doubts have been cast on the very existence of a tertiary phase in yaws, but deeply excavated ulcers and bone lesions are of far too frequent an occurrence in natives of the endemic zones to be a purely accidental occurrence. The photographs herewith reproduced (Pl. XXXI) illustrate some of the more typical lesions.

ACUTE PERIOSTITIS

An acute localized swelling situated on the anterior border of the tibia was met with in a Sinhalese who has been treated for generalized yaws for some considerable time previously. The swelling was hot and exquisitely tender, the skin tense and polished, indicating an acute inflammation of the periosteum (Pl. XXXI, fig. 1).

CHRONIC PERIOSTITIS

After subsidence of this acute stage, hard bony periosteal nodes remain; these are often of a considerable size, and are generally situated on the anterior border of the tibia or less commonly on the lateral borders of the radius and ulna.

OSTEITIS

A diffuse yaws osteitis which may result in a sabre-shaped deformity of the tibia, and often of the bones of the forearm as well, is exceedingly frequent. I saw one case in which there was, as a result of this process, a marked lateral deflection of the forearm; unfortunately I was unable to obtain a photograph of the particular case. A deformity of the lower extremities of this nature is shown in the smaller subject in fig. 3. A diffuse thickening of the clavicle is more rarely met with, a condition I had previously noted in Fiji in 1910. Apparently a rarefying process occurs in the centre of the long bones, thus rendering them especially liable to spontaneous fracture with consequent mal-union.

EPIPHYSITIS

The epiphyses of the phalanges and metacarpals are also attacked, and deformities of the hands and shortening of the digits as a result of this are extraordinarily common (fig. 2).

SYNOVITIS

A synovitis analogous to that of tertiary syphilis and often leading to total disorganisation of the knee-joint and generally associated with large cutaneous gummata in other portions of the body, are commonly seen in heavily infected districts (fig. 3).

ULCERATION

Excavated ulcers evidently of a gummatous nature are common amongst these natives, and there appears to be no valid reason for regarding them as the result of a separate infection. I saw one boy in whom there was a general circinate cutaneous ulceration obviously associated with bone lesions. In other cases these ulcers are so deeply excavated that the cicatricial tissue extends down to the bone and sometimes involves the whole circumference of the leg; a lymph stasis may thus result and an elephantoid condition of the leg ensue (fig. 4). There is no evidence that this form of elephantiasis is of filarial origin.

Yaws ulcerations on the dorsum of the foot and heel are especially prone to sinus formation; the resulting condition closely resembles mycetoma. Apart from the absence of streptothrix elements in the discharged pus there can be little doubt in determining a diagnosis, as the condition rapidly improves under salvarsan treatment.

Tertiary yaws ulcerations resulting in great hypertrophy of the lower lip (macrochelia) is also but rarely met with in subjects of a generalized infection.

GANGOSA

Lastly, I have to consider the ulceration of the nares frequently progressing to destruction of the septum and soft tissues of the nose. I can see no valid reason for separating, on clinical grounds alone, this lesion as a specific disease—'gangosa'—distinct from yaws.

Fig. 5 illustrates this condition well, emphasizing, as it does, the amount of cicatricial tissue on the face surrounding the lesion. A partial loss of the nose is common enough amongst the Sinhalese in the zones where yaws is endemic, but is not met with in other parts of the island where that disease is uncommon. Fijians are also commonly mutilated in this manner, and I have already mentioned that they are almost universally infected with yaws.

REFERENCES

BAHR, P. H. (1912). Filariasis and Elephantiasis in Fiji. Supplement No. I. Journ. Lond. Sch. Trop. Med., 1912, p. 4.

CASTELLANI, A. (1907). Experimental Investigations on Framboesia tropica (Yaws). Journ. Hyg., Vol. VII, pp. 558-569 Two plates.

EXPLANATION OF PLATE XXXI

Fig. 1. **Acute periostitis of the tibia.**

Fig. 2. **Epiphysitis of the phalanges and metacarpals, showing resulting deformity**

Fig. 3. Synovitis of the knee joint. Deformity of lower extremities in smaller subject.

Fig. 4. **Elephantoid condition of the leg due to lymph stasis produced by the cicatricial tissue resulting from framboesial ulcers.**

Fig. 5. **'Gangosa.'** Ulceration of the nares.

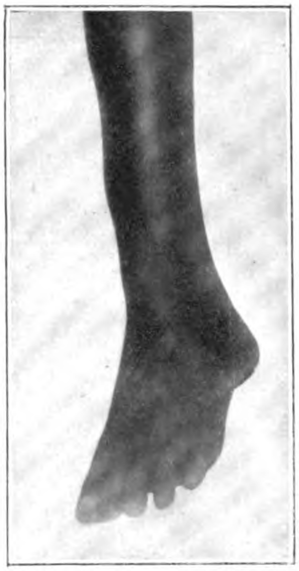


FIG. 1

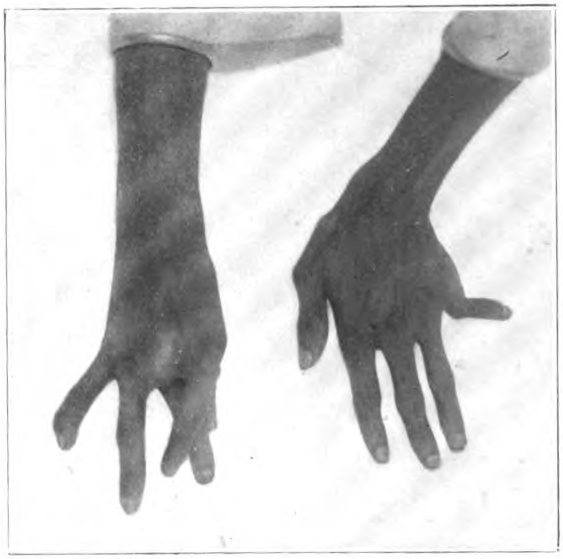


FIG. 2

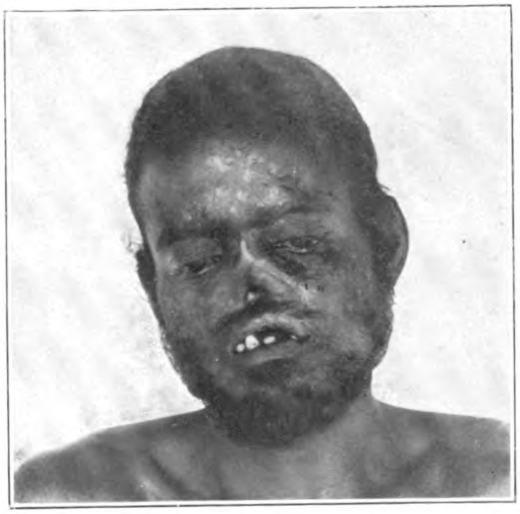


FIG. 5

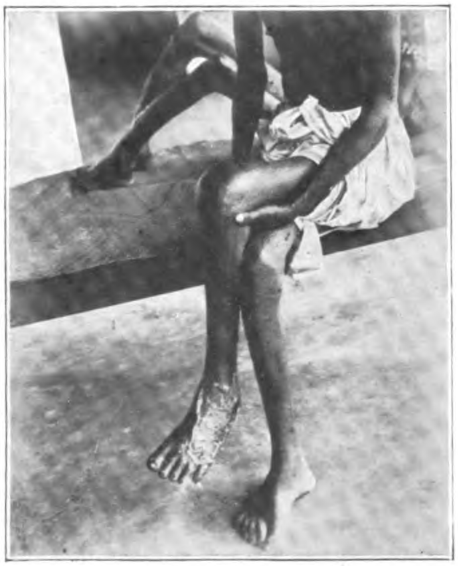


FIG. 3

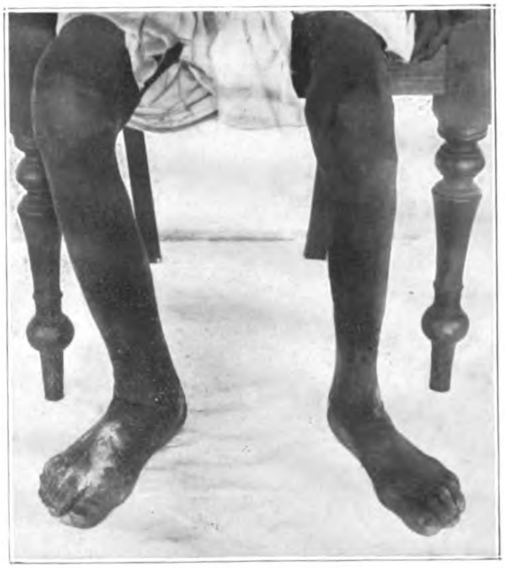


FIG. 4

C. Tinling & Co., Ltd., Imp.

INDEX

INDEX

PAGE
INDEX OF AUTHORS iii
GENERAL INDEX iii
INDEX OF SPECIES NEW TO SCIENCE ix

INDEX OF AUTHORS

PAGE
Bahr, P. H. 675
Barratt, J. O. Wakelin; and Yorke, W. 509
Blacklock, B.; and Yorke, W. ...1, 51, 55
Breinl, A.; and Priestley, H. ...565, 591
Breinl, A.; and Young, W. J. 575
Christopherson, J. B. 129, 485
Connal, A.; and Seidelin, H. 81
Deeks, W. E. 321, 353, 357
Eakin, W. C. W. 411
Fantham, H. B. 471
Fantham, H. B.; and Porter, A. ... 623
Gallagher, G. H.; and Macfie, J. W. Scott 379
Hatori, J. 537, 641
James, W. M. 133
Johnstone, J. E. L. 73
Johnston, J. E. L.; and Macfie, J. W. Scott 41
Johnston, T. Harvey 105
Lloyd, Ll. 83
Macfie, J. W. Scott 29, 417, 439
PAGE
Macfie, J. W. Scott; and Gallagher, G. H. 379
Macfie, J. W. Scott; and Johnston, J. E. L. 41
Marzinowsky, E. L. 367
Nicoll, W. 609
O'Brien, J. M. 369
Porter, A.; and Fantham, H. B. ... 623
Priestley, H. 113
Priestley, H.; and Breinl, A. ...565, 591
Schwetz, J. 497
Seidelin, H. 553
Seidelin, H.; and Connal, A. 81
Stephens, J. W. W. 119, 639
Taylor, J. A. 13
Thomson, D. 19, 85
Todd, J. L. 469
Whyte, G. Duncan 659
Yorke, W.; and Barratt, J. O. Wakelin 509
Yorke, W.; and Blacklock, B. ...1, 51, 55
Young, W. J.; and Breinl, A. 575

GENERAL INDEX

PAGE
Acanthocephala in Queensland 110
Acanthocheilonema perstans...73, 75, 78, 79
Acanthotaenia (*Ophiotaenia*) *striata*, n.sp. 108
Acanthotaenia spp. 109
Africa. Plasmodia in monkeys in West 81
Agama colonorum, blood parasites of... 455
Amoebic dysentery, Aetiology of ... 325
„ „ due to *Entamoeba histolytica* 325
„ „ Entamoebae in... 134
„ „ Pathology of ... 328
„ „ Symptomatology of 330
„ „ Treatment of ... 333
„ „ Treatment of, with emetine 353
Anglo-Egyptian Sudan. Naso-oral leishmaniasis in 485
PAGE
Anti-malarial campaign in Formosa... 537
Antimony trioxide in trypanosomiasis 55
Arneth's neutrophile changes 565
Atoxyl in fowl spirochaetosis 44
Auchmeromyia luteola, biology of larva, nymph and imago 499
„ „ morphology ... 498
Babesia bigemina (?) in Nigeria 443
„ in dwarf cattle—no symptoms 441
„ in domestic animals in Nigeria 439
„ sp. in Hausa cattle 443
Bacillary dysentery in Panama 345
Bacilliform bodies in rat red blood corpuscles 450
Bahr, P. H. Notes on Yaws in Ceylon with special reference to its distribution in that island and its tertiary manifestations 675

PAGE
Balantidium dysentery in Panama ... 347
Bancroftiella glandularis 108
Banti's disease 660
Barratt, J. O. Wakelin, and Yorke, W. The relation of bile pigments to haemoglobin 509
Bed bugs and disease germs 19
Bile pigments in relation to haemoglobin 509
„ Technique of obtaining........... 510
Bilharzia dysentery in Panama 347
Birds, scratching, and *Glossina* pupae 83
Bismuth subnitrate in amoebic dysentery 334
Blacklock, B., and Yorke, W. Antimony trioxide in the treatment of experimental trypanosomiasis 55
Blacklock, B., and Yorke, W. Observations on a so-called cure for trypanosomiasis 51
Blacklock, B., and Yorke, W. The differentiation of the more important mammalian trypanosomes ... 1
Blackwater fever, defects in reports... 639
,, ,, in Formosa 641
,, ,, Formosa, mortality 654
,, ,, Formosa, racial relationship ... 649
,, ,, Formosa, seasonal prevalence 650
,, ,, Malarial parasites in 652
,, ,, Quinine as immediate cause of 653
,, ,, recurrence 654
,, ,, schedule for reporting cases 640
Blood conditions of children in tropical Australia............... 591
„ neutrophile changes of Arneth 565
„ parasites in Nigeria 439
Bombus spp., infected with *Nosema bombi* 624
Breinl, A., and Priestley, H. Changes in the neutrophile blood picture of Arneth observed in children living in tropical Queensland 565
Breinl, A., and Priestley, H. Observations on the blood conditions of children of European descent residing in tropical Australia 591

PAGE
Breinl, A., and Young, W. J. The occurrence of lead poisoning amongst North Queensland children 575
Bronchial spirochaetosis in Uganda... 13
Bufo regularis, blood parasites of...... 460
Carcinoma, Bed bugs fed on case of 27
Cestoda in Queensland 106
Ceylon, Yaws in 675
Children of Tropical Queensland, Arneth neutrophile changes 565
Blood conditions of 591
Lead poisoning among 575
China, South, Splenomegaly in 659
Christopherson, J. B. On a case of naso-oral leishmaniasis (corresponding to the description of Espundia); and on a case of Oriental sore, both originating in the Anglo-Egyptian Sudan 485
Christopherson, J. B. The mutilation of wounded on the battlefield: a case described 129
Chronic lymphatic leukaemia, Bed bugs fed on case of.................... 26
Chrysops dimidiata in N. Nigeria...... 382
Cimex lectularius, Organisms normally found in 21
,, ,, Feeding of, on patients............ 23
Congo floor maggot, Biology of 497
Connal, A., and Seidelin, H. A note upon the occurrence of a Plasmodium in the blood of West African monkeys......................... 81
Crescents, Malarial, origin and development 86, 100
Davainea sphecotheridis, n.sp. 106
Deeks, W. E. Dysenteries in the Canal Zone, with special reference to amoebic dysentery 321
Deeks, W. E. Emetine in amoebic dysentery 353
Deeks, W. E. Six day fever in Panama 357
Differentiation of mammalian trypanosomes 1
Dirofilaria immitis 73
Diphtheric colitis in Panama 350
Dumb-bell shaped bodies in lizards' blood....................................... 457
Dysentery in Panama Canal Zone ... 321

PAGE
Dysentery in Panama Canal Zone, Entamoebae associated with, 168, 180, 254, 265
Dysentery in Panama Canal Zone, Various types of ... 322, 351
Eakin, W. C. W. Notes and observations on Sleeping Sickness in the Eket district (Appendix I to Macfie and Gallagher, p. 379) ... 411
Echinorhynchus, spp. ... 110
Ecuador, Yellow fever in ... 369
Eket, physical features ... 380
  Sleeping sickness in ... 29, 379, 411
Emetine in amoebic dysentery ... 353
Entamoebae, Bibliography of human intestinal ... 305
  ,, chromidia formation (so-called) ... 275
  ,, Classification of human ... 253
  ,, Correlation of the types, in dysentery ... 270
  ,, Human, in Panama ... 133
  ,, morphology ... 168
  ,, Relation of dysenteries to types of ... 269
  ,, Summary of literature on 136
  ,, technique ... 282
Entamoeba coli ... 200
  ,, ,, Cyst formation of ... 231
  ,, ,, cytoplasm and motion 206
  ,, ,, Distribution of ... 203
  ,, ,, Encystment of ... 239
  ,, ,, free nuclei and enucleate amoebae ... 228
  ,, ,, Multiplication of ... 222
  ,, ,, Nucleus of ... 213
  ,, ,, Size of ... 203
  ,, ,, Species resembling ... 251
Entamoeba histolytica ... 134
  ,, ,, and *E. tetragena*, Discussion of 254
  ,, ,, and *E. tetragena*, same organism ... 134, 322
Entamoeba (*tetragena*) *histolytica*, chromidia and cyst formation ... 156
  Degeneration forms of ... 154
  Development of ... 151
  Morphology of ... 151
  synonymy ... 134
  Vegetative forms of ... 151

PAGE
Entamoeba tetragena ... 151, 177
Espundia in Anglo-Egyptian Sudan ... 485
Fantham, H. B. The granule phase of Spirochaetes ... 471
Fantham, H. B., and Porter, A. The morphology, biology and economic importance of *Nosema bombi*, n.sp., parasitic in various humble bees (*Bombus* spp.) ... 623
Filaria demarquayi ... 73
Filarial embryos in lizards, Nigeria ... 456
  ,, ,, toads, Nigeria ... 462
Filariidae in man ... 73
Formosa, Anti-malarial campaign in ... 537
  ,, Blackwater fever in ... 641
  ,, Malaria in ... 642
Fowls, disease characterised by leucocyte inclusions ... 451
  ,, spirochaetosis blood changes ... 43
  ,, ,, in S. Nigeria ... 41
  ,, ,, post-mortem ... 42
  ,, ,, symptoms ... 42
Gallagher, G. H., and Macfie, J. W. Scott. Sleeping sickness in the Eket district of Nigeria ... 379
Gambian mice, trypanosome of ... 469
Gangosa ... 679
Glossina, Anatomical parts affected by trypanosomes ... 10
  ,, as hosts of trypanosomes ... 9
  ,, Incubation period of trypanosomes in ... 10
  ,, Morphology of trypanosomes in ... 10
  ,, pupae and scratching birds ... 83
  ,, spp., in N. Nigeria ... 382
  ,, *tachinoides*, possible transmitter of *T. nigeriense* ... 407
  ,, ,, Spirochaete in gut of ... 464
  ,, ,, Trypanosomes in wild ... 417
Granules in Spirochaetes ... 471
Guayaquil (Ecuador), Yellow fever in 369
Guinea-pig, *Klossiella* in kidney of ... 553
  ,, *Paraplasma cobayae*, n.sp., in ... 446
  ,, ,, *flavigenum*, in 445
  ,, Spirochaete in blood of ... 447
Gyrocoelia, sp. ... 108

PAGE

Haemoglobin, Relation of bile pigments to 509
,, Preparation of 511
Haemocystidia in lizards, Nigeria ... 459
Haemogregarina tunisiensis (?) in toads, Nigeria 463
Haemogregarines in lizards, Nigeria... 458
,, toads, Nigeria ... 463
Hatori, J. Blackwater fever in Formosa 641
Hatori, J. The anti-malarial campaign in Formosa 537
Hodgkins' disease, bed bugs fed on case of 26
Humble bees, infected with *Nosema bombi*, n.sp. 624
Inclusions in leucocytes of fowls, Nigeria 451
James, W. M. A study of the Entamoebae of man in the Panama Canal Zone 133
Johnston, J. E. L. Observations on variations in form of microfilariae found in man 73
Johnston, J. E. L., and Macfie, J. W. Scott. A note on the occurrence of spirochaetosis of fowls in Southern Nigeria 41
Johnston, T. Harvey. Second report on the Cestoda and Acanthocephala collected in Queensland 105
Klossiella cobayae, n.sp. 560
,, *muris* 553
Larva of *Auchmeromyia luteola* ...497, 500
,, *Onchocerca gibsoni*, Migration of 609
Lead poisoning amongst N. Queensland children 575
,, symptoms in young and old 583
Leishmaniasis, naso-oral in Sudan ... 486
,, oriental sore 487
Leishmania tropica, var. *americana* ... 490
Leprosy, nodular, bed bugs fed on case of 24
Leucocytes, inclusions in, of fowls, Nigeria 451
Leucocytogregarina in lizards, Nigeria 459
Leukaemia, Bed bugs fed on cases of... 26
Lizards, Blood parasites of Nigerian... 454
,, Dumb-bell shaped bodies in blood......................... 457

PAGE

Lloyd, Ll. Note on scratching birds and tsetse 83
Loa loa 75
Lymphadenoma, Bed bugs fed on case of 26
Macfie, J. W. Scott. Notes on some blood-parasites collected in Nigeria 439
Macfie, J. W. Scott. On the pathogenicity of the trypanosome (*T. nigeriense*) from a case of sleeping sickness from Eket, Southern Nigeria 29
Macfie, J. W. Scott. Trypanosomes found infecting wild *Glossina tachinoides* (Appendix II to Macfie and Gallagher, p. 379) 417
Macfie, J. W. Scott, and Gallagher, G. H. Sleeping sickness in the Eket district of Nigeria 379
Macfie, J. W. Scott, and Johnston, J. E. L. A note on the occurrence of spirochaetosis of fowls in Southern Nigeria 41
Malaria, Bed bugs fed on case of ... 27
,, Communication from Commission for study of malaria in Russia 367
,, malignant tertian, crescents 85
,, in Formosa 537, 642
,, Quinine treatment of ...546, 647
,, prevention, regulations for... 547
Malarial dysentery in Panama 348
,, parasite in autopsy smears of organs 92, 100
,, ,, crescents in peripheral blood...86, 100
,, ,, crescents, origin and development of... 85
,, ,, microgametes, flagellation of 97, 101
,, ,, a new 119
Mammalian trypanosomes, differentiation of 1
Marzinowsky, E. L. Communication from the Commission for the study of malaria in Russia 367
Mice, trypanosome of Gambian 469
Microfilariae found in man 73
,, human, in N. Nigeria... 74
,, Variations in form of human 74
Microfilaria philippinensis 73

PAGE

Microgametocytes, malarial, flagellation of ... 97
Microsporon scorteum, n.sp., from case of ringworm 113, 117
,, ,, cultural characters ... 114
,, ,, morphology ... 115
Monkey, West African, Plasmodia in 81
Mutilation of wounded on battlefield 129
Naso-oral Leishmaniasis in Sudan...... 485
Nephritic dysentery in Panama 348
Neutrophile changes of Arneth in children ... 565
Nicoll, W. On the migration of the larvae of *Onchocerca gibsoni* through the capsule of the worm nodule...... 609
Nigeria, Blood parasites collected in... 439
,, (Eket), Sleeping sickness in 379
,, ,, *T. nigeriense* from a case of sleeping sickness in ... 29
,, (Southern), Spirochaetosis of fowls in ... 41
Nosema bombi, n.sp., Economic importance of 633
,, ,, ,, Effects of......... 624
,, ,, ,, Hosts of 624
,, ,, ,, Life history of... 626
,, ,, ,, mode of infection 632
,, ,, ,, Morphology of... 628
,, ,, ,, Preventive measures against 634
Nymph of *Auchmeromyia luteola* 505
O'Brien, J. M. A study of some cases of yellow fever in Guayaquil, Ecuador ... 369
Onchocerca gibsoni, Biology of 609
,, ,, migration of larvae through nodule capsule 614
,, *volvulus* ... 73
(*Ophiotaenia*) *Acanthotaenia striata*, n.sp. ... 108
Oriental sore in Sudan ... 487
Pathogenicity of *T. nigeriense* 29
Panama Canal Zone, Dysenteries in... 321
,, ,, ,, Entamoebae of man in......... 133
,, ,, ,, Six-day fever in 357
Paraplasma cobayae, n.sp., in guinea-pigs, Nigeria 446

PAGE

Paraplasma flavigenum (?) in guinea-pigs, Nigeria 445
Parasites in blood, Nigeria 439
Pellagra and dysentery, Panama 350
Plasmodia in monkeys ... 81
Plasmodium tenue, n.sp. ... 122
Pneumonic symptoms with bronchial spirochaetosis ... 13
Polymorphonuclear leucocytes in yellow fever ... 371
Porter, A., and Fantham, H. B. The morphology, biology and economic importance of *Nosema bombi*, n.sp., parasitic in various humble bees (*Bombus* spp.) ... 623
Priestley, H. *Microsporon scorteum*, n.sp., from a case of ringworm in man ... 113
Priestley, H., and Breinl, A. Changes in the neutrophile blood-picture of Arneth observed in children living in tropical Queensland ... 565
Priestley, H., and Breinl, A. Observations on the blood conditions of children of European descent residing in tropical Australia 591
Pupa of *Auchmeromyia luteola* 501
Queensland, Blood conditions in children in 591, 608
,, Cestoda and Acanthocephala of ... 105
,, Lead poisoning amongst children in... 575
,, Neutrophile changes of Arneth in children of tropical...565, 573
Quinine as immediate cause of blackwater ... 653
,, in treatment of malaria...546, 647
Rhodesia, N., Guinea fowl and *Glossina* pupae in ... 83
Rat, Bacilliform bodies in red blood cells ... 450
Ringworm, *Microsporon scorteum*, n.sp., from a case of... 113
Russia, Communication from the Malaria Commission ... 367
Salvarsan in fowl spirochaetosis 44
Sarcoma, Bed bugs fed on case of...... 27
Schwetz, J. Quelques observations préliminaires sur la morphologie et la biologie de la nymphe et de l'image de l'*Auchmeromyia luteola*, Fabr... 497

PAGE
Scratching birds and *Glossina* pupae... 83
Seidelin, H. *Klossiella* sp. in the kidney of the guinea-pig 553
Seidelin, H., and Connal, A. A note upon the occurrence of a Plasmodium in the blood of West African monkeys........................ 81
Six day fever in Panama 357
,, physical signs 358
,, rash 360
,, symptomatology 358
Sleeping sickness in Eket, Nigeria, 379, 411
,, ,, ,, Aetiology of 383
,, ,, ,, blood examination ... 390
,, ,, ,, Diagnosis of 390
,, ,, ,, gland palpation 393
,, ,, ,, influence of sex and age......... 384
,, ,, ,, symptoms.... 396
,, ,, ,, *T. nigeriense* in 403
,, ,, ,, treatment with atoxyl ... 402
Sparganum sp............................ 110
Spirochaeta bronchialis.................... 17
,, ,, Granules in, 473, 480, 483
,, *gallinarum* 46
Spirochaetes, Culture of, from blood of guinea-pig 447
,, in gut of *Glossina tachinoides* 464
,, Granule phase of 471
Spirochaetosis, Bronchial, in Uganda 13
,, of fowls, S. Nigeria ... 41
,, ,, Parasites of... 46
,, ,, symptoms ... 42
,, ,, treatment with atoxyl and salvarsan 44
Spleno-medullary leukaemia, Bed bugs fed on case of 26
Splenomegaly, blood examination ... 666
,, in South China 659
,, symptoms 662
,, Various causes of 659

PAGE
Stephens, J. W. W. A new malaria parasite of man 119
Stephens, J. W. W. Studies in blackwater fever. II. A schedule for recording cases of blackwater fever 639
Sudan, Anglo-Egyptian, Leishmaniasis in 485
Swatow, Splenomegaly in 659
Tabanidae in N. Nigeria 382
Taylor, J. A. Bronchial spirochaetosis in Uganda, with pneumonic symptoms................................ 13
Technique for Entamoebae 282
Tertiary manifestations of Yaws 675
Tetrarhynchus sp. 110
Theileria parva (?) in cattle in Nigeria 441
,, Cultivation of 442
Thomson, D. Attempts to find disease germs in the European bed bug (*Cimex lectularius*) after feeding experiments in various diseases—Leprosy, Lymphadenoma, Carcinoma, etc. 19
Thomson, D. The origin and development of Gametes (Crescents) in malignant tertian malaria: some observations on flagellation, etc.... 85
Ticks, Various granules found in ... 475
Toads, Blood-parasites of Nigerian 454, 460
Todd, J. L. The trypanosome of Gambian mice 469
Treatment of trypanosomiasis with antimony trioxide 55
Trypanosoma acomys in Gambian mice 470
T. equiperdum, characteristic plaques of 8
T. mega in toads, Nigeria 460
T. nigeriense, Invertebrate host of ... 406
,, Measurements of 405
,, Pathogenicity of ...29, 403
,, Possible reservoir of...... 409
T. pecaudi (*T. ugandae*) in wild *Glossina tachinoides* 417, 418
T. pecorum in domestic animals 409
,, in wild *Glossina tachinoides* 417
,, *T. nanum* and *T. congolense* probably varieties of same species 8, 425
T. rotatorium in toads, Nigeria 460
T. vivax in wild *Glossina tachinoides* 417
,, in domestic animals 409
Trypanosomes, anterior station of ... 10

PAGE
Trypanosomes, Biometric curves and diagnosis of............ 5, 7
,, Differentiation of mammalian 1
,, flagellum 2
,, in domestic animals, Nigeria 409
,, Incubation period in Glossina 10
,, infecting wild *Glossina tachinoides* 417
,, in lizards, Nigeria ... 455
,, in toads, Nigeria 460
,, invertebrate host of... 9
,, Mammalian 1
,, Pathogenicity of 7, 36
,, qualitative characters 2
,, quantitative characters 5
,, Serum diagnosis of ... 9
,, Tabular list of...*facing* 11
Trypanosomiasis treatment, so-called cure for 51
,, ,, with antimony trioxide 55
Tsetse-fly and scratching birds 83
Tubercular dysentery in Panama...... 348
Uganda, Bronchial spirochaetosis in... 13
West Africa, Plasmodia in monkeys in 81
Whyte, G. Duncan. Splenomegaly: Being a report on a hundred and four cases met with in South China 659
Wounded, mutilation of, on battlefield 129

PAGE
Yaws in Ceylon 675
,, ,, epiphysitis in 679
,, ,, osteitis in 678
,, ,, periostitis in 678
,, ,, synovitis in 679
,, ,, tertiary manifestations in 677
Yellow fever in Ecuador 369
,, blood examinations of... 371
,, diagnosis of 376
,, immunity of infants to 373
,, polymorphonuclear leucocytes in 371
,, symptoms 369
,, treatment 376
Yorke, W., and Barratt, J. O. Wakelin. The relation of bile pigment to haemoglobin 509
Yorke, W., and Blacklock, B. Antimony trioxide in the treatment of experimental trypanosomiasis 55
Yorke, W., and Blacklock, B. Observations on a so-called cure for trypanosomiasis............................ 51
Yorke, W., and Blacklock, B. The differentiation of the more important mammalian trypanosomes... 1
Young, W. J., and Breinl, A. The occurrence of lead poisoning amongst North Queensland children 575

INDEX OF SPECIES NEW TO SCIENCE

PAGE
Acanthotaenia (*Ophiotaenia*) *striata* ... 108
Davainea sphecotheridis 106
Klossiella cobayae 560
Microsporon scorteum 113, 117

PAGE
Nosema bombi 623, 624, 631
Paraplasma cobayae....................... 446
Plasmodium tenue 122

C. TINLING AND CO., LTD.
PRINTERS TO THE UNIVERSITY PRESS OF LIVERPOOL
VICTORIA STREET

Date Due

31 [illegible]

-1 APR '65 HML

-6 APR '65 HML

Demco 293-5

3 2044 081 513 632